Study of Phytochemical, Elemental Analysis and Antioxidant Activities on the Leaves of *Carica papaya* L.

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ABSTRACT

Carica papaya L. belongs to the family Caricaceae. *Carica papaya* L. were collected from Kyaukse township, Mandalay Region. Morphological, phytochemical, elemental analysis and antioxidant activities of *Carica papaya* L. were carried out, to get their correct identification. In morphological study, *Carica papaya* L. was perennial herbs and stem are unbranched, cylindrical. Leaves are simple, palmately lobed and inflorescence are axillary. Staminate flower and pistillate flower are unisexual and actinomorphic. Fruit is ellipsoid. Seed are many seeded and black. In the phytochemical studies in the leaves of *Carica papaya* L. showed the presence of a wide of secondary metabolites such as alkaloid, glycoside, flavonoid, phenol, polyphenol, saponin, tannin, and steroid. Lipophenol and reducing sugar are absent. From EDXRF data, psotassium, magnesium, calcium, sulfur, phosphorous, iron, manganese, strontium, titanium, zinc were present. Antioxidant activities of ethanol extract of papaya are investigated by DPPH free radical scavenging method. IC_{50} values of ethanol extract were 4.37 µg/mL.

Keywords : *Carica papaya* L., morphological, phytochemical, elemental analysis, antioxidant activities

1 INTRODUCTION

Papaya which belongs to the family Caricaceae originated in Southern Mexico and neighbouring Central America. Now it exists in every tropical and subtropical countries, while it tolerates dry and hot environmental adoptable for diverse soil types. Papaya was introduced to Sri Lanka in the 16th century by the Portuguese (Srikantha *et al*. 2001).

Carica papaya L. belongs to the family caricaceae with over twenty species but only one member of the genus Carica is cultivated as a fruit tree, ornamentals. *Carica papaya* L. leaves have been used in the treatment of various ailments including urinary tract infections. The leaves of papaya plant contain karpain, a substance that kills microorganisms that often interfere with the digestive function (Alorkpa *et al*. 2016).

Carica papaya L. is usually cultivated in tropical regions, its commonly called as papaya in this part of the world. *Carica* is the only genus in the family Caricaceae. It belongs to group of

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plants known as Laticiferous plants (Vays *et al*. 2014).

Young leaves are rich in flavonoids, alkaloids, phenolic compounds found in leaves. Both leaf and fruit of the *Carica papaya* L. possess carotenoids namely beta-carotene. If possess medicinal properties like anti-inflammatory, antifertility, abortifacient, hepatoprotective, wound healing and antitumor (Anjum *et al*. 2013).

The papaya tree is belonging to family Caricaceae having four genera in world. The genus *Carica papaya* L. is represented by four species in India, of which *Carica papaya* L. is most widely cultivated and best-known species. The fruit, leaves and latex obtain from papaya plant used medicinally and for various other purposes. Papain, major chemical compound extracted from fruit and stem latex is used in brewing and wine making and in textile and tanning industry (Jean Bruneton 2001).

Leaves contain large amounts of alkaloids, carpaine and pseudocarpine which create positive effects on hearts as well as on respiration. Leaf extract of *Carica papaya* L. is well known as anti-tumor agent. The papaya fruit, as well as other parts of the plant, contain a milky juice in which an active principle known as papain. Phytochemicals are compounds that occur naturally in plants. Pawpaw plants produce natural compounds in leaf that possess both highly anti-tumor and pesticidal properties. Fresh green pawpaw leaf possess antiseptic properties, dried leaf can serve as a tonic and blood purifier. The green unripe pawpaw has therapeutic value due to its antiseptic quality (Eleazu 2012).

The term “antioxidant” is mostly used for two entirely different groups of substances: industrial chemicals that are added to products to prevent oxidation, and naturally occurring compounds that are present in foods and tissue. The former, industrial antioxidants, have diverse uses: acting as preservatives in food and cosmetics, and being oxidation-inhibitors in fuels.

Importantly, antioxidant dietary supplements have not yet been shown to improve health in humans, or to be effective at preventing disease. Supplements of beta-carotene, vitamin A and vitamin E have no effect on mortality rate or cancer risk. Additionally, supplementation with selenium or vitamin E do not reduce the risk of cardiovascular disease.

The aim of this research is to study the medicinal value of plant drugs. The objectives are to investigate the plant constituents and their action. The objective of this research was to study the effect of altitude and different shading conditions on the vegetative growth of *Carica papaya* L. to determine the best cultivation practice of this plants.

2 MATERIALS AND METHODS

2.1 Collection, Identification and Preparation of *Carica papaya* L.

The specimens of *Carica papaya* L. were collected from Kyaukse district, Mandalay region during the flowering period on 2017. The collected male papaya plants were taxonomically identified with the help of references such as Hooker 1885 and Dassanayake 1987. The fresh specimens were pressed, dried and preserved for morphological studies. Herbarium specimens were prepared and kept in the Herbarium of Botany Department, Kyaukse University.

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2.2 Preliminary Phytochemical Characterization of *Carica papaya* L.

Preliminary phytochemical investigation was carried out at Department of Chemistry, University of Mandalay, according to Harbone 1984. It was carried out for the male papaya leaves with a view to determine the constituents of alkaloid, glycoside, flavonoid, phenol, polyphenol, lipophenol, reducing sugar, saponin, tannin and steroid.

2.3 Elemental Analysis by Using Energy Dispersive X-rays Fluorescence

Elemental analysis was carried out by EDXRF method at Department of Physics, University of Mandalay. Dried and cleaned male papaya leaves were weighed and the pre-ash solutions were carried out on the sand bath until all the combustible materials were burnt. The pre-ash samples were then placed inside the electric muffle furnace and heated gradually raising the temperature until 450˚C. The process of heating, cooling and weighing were repeated until constant weight of the ash samples were obtained. About 2.5 g of ash samples were fabricated into pellet for EDXRF spectrometry.

2.4 Determination of Antioxidant Activity

In this experiment, 1-1 diphenyl-2- picryl-hydrazyl (DPPH) powder was used as stable free radical. Ascorbic acid was used as standard antioxidant and ethanol was used as solvent. The absorbance was determined at 517 nm wavelength.

2.5 DPPH Assay

DPPH free radical method is an antioxidant assay based on electron- transfer that produces a violet solution in ethanol.

This free radical, stable at room temperature, is reduced in the presence of an antioxidant molecule, giving rise to colorless ethanol solution.

The use of DPPH assay provides an easy and rapid way to evaluate antioxidants by spectrophotometry.

2.6 Preparation of Reagents

In this experiment three solutions were prepared. They are DPPH solution, standard solution and various concentration of sample solution.

2.7 Preparation of DPPH Solution

2.346 mg of DPPH powder was dissolved in 100 mL of ethanol. This solution was thoroughly mixed at room temperature and it was stored in brown colored flash. This solution kept for no longer than 24 hours.

2.8 Preparation of Standard Solution

2 mg of ascorbic acid was dissolved in 20 mL of ethanol. This solution was thoroughly mixed at room temperature to obtain 100 μg/mL of standard solution. The concentration of standard solution (50, 25, 12.5, 6.25 and 3.124 μg/mL) was determined by using two fold

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dilution methods. 1 mL of ascorbic acid and 2 mL of DPPH solutions were thoroughly mixed for about 15 min at room temperature. The absorbance of mixture was measured at 517 nm.

2.9 Preparation of Test Sample Solution

2 mg of ethanol crude extract of dried male papaya leaves was dissolved in 100 mL of ethanol. This solution was thoroughly mixed at room temperature for 15 minutes to obtain 20 μg/mL of sample solution. The concentration of test sample solutions (20, 16, 12, 8 and 4 μg/mL) were prepared by serial dilution method.

2.10 Determination of Absorbance of Sample Solution

1 mL of sample solution and 2 mL of DPPH solutions were thoroughly mixed for about 15 minute at room temperature. The absorbance of the mixture was measured at 517 nm.

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

This formula is the calculation of percent inhibition of (IC_{50}) value. The half maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness of a substance in inhibiting a specific biological or biochemical function.

2.11 Determination of Half Maximal Inhibitory Concentration

(IC_{50}) values were obtained from the best-fit line plotted concentration verses inhibition.

3 RESULTS

3.1 Morphological Studies of *Carica papay* L.

Scientific name - *Carica papaya* L.
Family - Cariaceae
Local name - Thinbaw
Flowering period - Throughout the year

Perennial herbs, erect, 2-4 m high, unbranched, trunk soft, marked with large petiole scar. Stem unbranched, cylindrical, white. Leaves palmately 7-9 lobed, arranged in spiral, 20- 32 long, 26- 50 cm wide; petioles stout, hollow, 18- 36 cm long, 1.0- 1.5 cm wide, pale green, glabrous, deeply lobe blade, acuminate at apex, base cordate, upper surface is green and lower surface is pale green, Inflorescence axillary, much branched cyme with many flowers; penduncle 18- 27 cm long. Staminate flower unisexual, actinomorphic, 2- 3 cm long, pale yellow; pedicles 1.5- 2.0 cm long, exerted. Corolla tube thin, slender, white. Filament filiform, short 1-2 mm long, pale yellow; anther dithecous, longitudinal dehiscent. Pistillate flowers unisexual, actinomorphic, hypogynous, yellow to whitish flower, axillary spikes or racemes: calyx 5 lobed. Petals 5, oblong, 6 cm long, 1 cm wide, yellow to whitish, glabrous. Carpels 5, syncarpous; ovary pale yellow, ellipsoid, superior, unilocular, with many ovules in the locules, parietal placentation; style slender, short; stigma five fan-shaped. Fruit ellipsoid, indehiscent, subglobose, obovoid or oblong- cylindrical many seeded.

Specimen examined **:** Kyaukse Township, Mandalay Region

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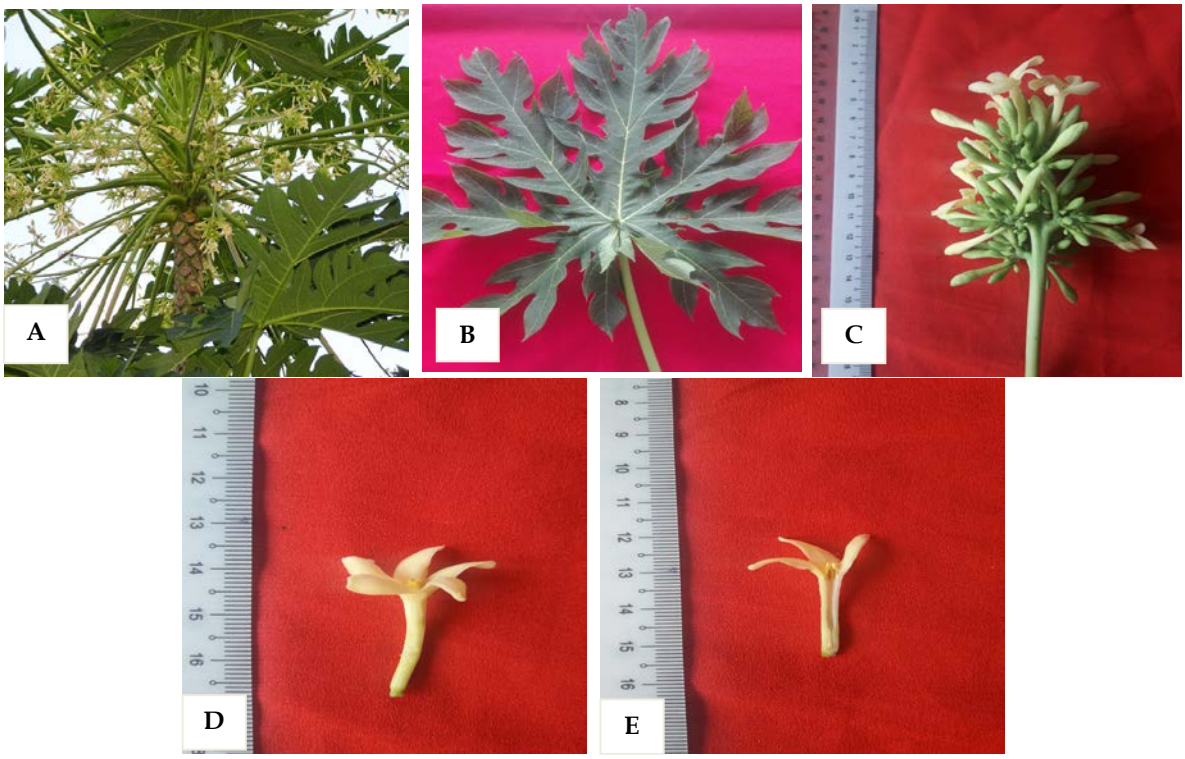

Figure 1. Morphological studies of in *Carica papaya* L.
A. Habit B. Male Leaves C. Male Inflorescence
D. Male Flower E. L.S of Flower

3.2 Preliminary Phytochemical Constituents of the Male Leaves of *Carica papaya* L..

Phytochemical screening were determined at Department of Chemistry, University of Mandalay, according to Harbone 1984. Preliminiary phytochemical properties were carried out for the male papaya leaves. With a view to determine the prescence of the constituent of alkaloid, glycoside, phenol, polyphenol, saponin, tannin and steroid. Lipophenol and reducing sugar are absent. The results were present in Table 1.

3.3 Elemenetal Analysis of the Male Leaves of *Carica papaya* L. by Using Energy Dispersive X-rays Fluorescence

The elements that contain in the male papaya leaves were analyzed by using Energy Dispersive X-rays Fluorescent (EDXRF) method at Department of Physics, Monywa University. Among the elements having atomic number 13 to 92, it was found that there are 10 elements including macro elements and micro elements samples. The elements having high to low concentration were present in Table 2-4 .

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Table 1. Preliminary Phytochemical Constituents in the Male Payaya Leaves

No	Constituents	Extract	Test Reagent	Observation	Result
1	Alkaloid	Water	Dragendroff's reagent Wagnar reagent	Reddish brown Reddish brown	+
2	Glycoside	Water	10%$(CH_3CO)_2$ Pb	Brick red ppt.	+
3	Flavonoid	Ethanol	Conc.HCl,Mg	Greenish ppt.	+
4	Phenol	Water	10%$FeCl_3$	Reddish brown	+
5	Polyphenol	Ethanol	1%$FeCl_3$,$K_3Fe(CN)_6$	Greenish solution	+
6	Lipophenol	Water	0.5N KOH	Yellow solution	_
7	Reducing Sugar	Water	Benedict's solution	Green solution	_
8	Saponin	Water	Shake	Froth	+
9	Tannin	Water	10%$FeCl_3$	Yellow solution	+
10	Steroid	Ethanol	$CHCl_3$,Conc.H_2SO_4	brownish yellow	+

(+) = present (-) = absent

Table 2. The Elements Present in the Male Papaya Leaves

No.	Elements	Concentration(%)
1	Potassium (K)	1.424
2	Magnesium (Mg)	1.276
3	Calcium (Ca)	1.070
4	Sulfur (S)	0.361
5	Phosphorus (P)	0.231
6	Iron (Fe)	0.015
7	Titanium (Ti)	0.002
8	Strontium (Sr)	0.002
9	Manganese (Mn)	0.002
10	Zinc (Zn)	0.001
11	C, H,O	95.614

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Table 3. The Macroelements Present in the Male Payaya Leaves

No	Macroelements	Concentration (%)
1	Potassiun (K)	1.424
2	Magnesium (Mg)	1.276
3	Calcium (Ca)	1.070
4	Sulfur (S)	0.361
5	Phosphorus (P)	0.231

Table 4. The Microelements Present in the Male Papaya Leaves

No	Microelements	Concentration (%)
1	Iron (Fe)	0.015
2	Manganese (Mn)	0.002
3	Strontium (Sr)	0.002
4	Titanium (Ti)	0.002
5	Zinc (Zn)	0.001

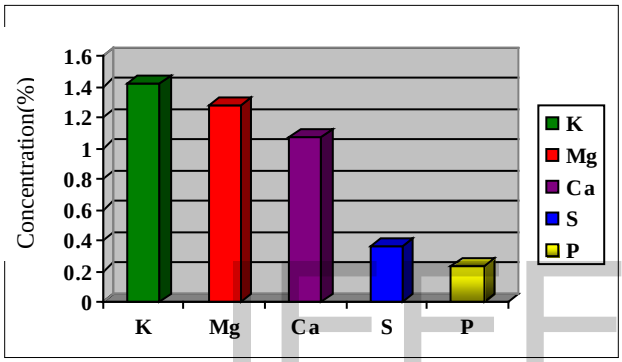

Figure 2. The Macroelements Present in the Male Papaya Leaves

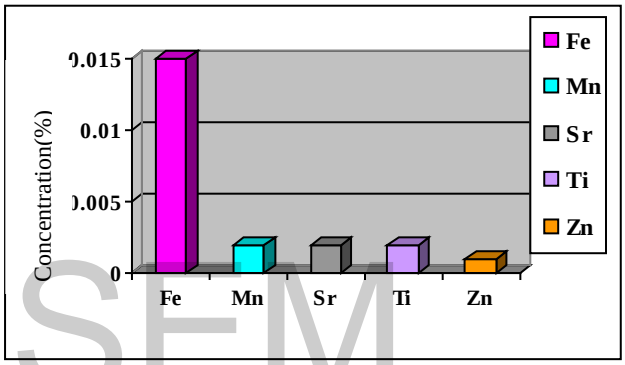

Figure 3. The Macroelements Present in the Male Papaya Leaves

3.4 The Antioxidant Activity Using DPPH Assay

The result of antioxidant activity using DPPH assay in standard ascorbic acid was shown in Table (5).

Table 5. % Inhibition of Various Concentration of Standard Ascorbic Acid

StandardConcentration (µg/mL)	Mean Absorbance	Mean % inhibition	IC_{50} (µg/mL)
50	0.260	58.7	41.9
25	0.426	32.4	
12.5	0.551	12.5	
6.25	0.614	2.5	
3.125	0.626	0.6	

Absorbance of control = 0.63

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IC_{50} value was calculated by using linear regressive equation.

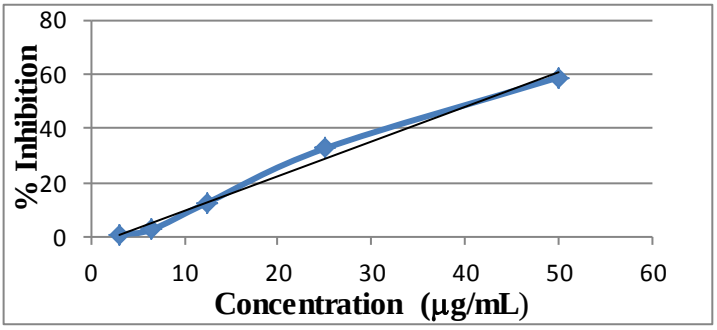

Figure 4. % Inhibition of Different Concentration of Standard Ascorbic Acid

The antioxidant activity of ethanol extract of sample was determined using DPPH assay and the results were shown in Table (6).

Table 6. % Inhibition of Various Concentration of Sample

Sample Concentration (mg/mL)	Mean Absorbance	Mean % Inhibition	IC_{50} (mg/mL)
4	0.364	42.2	4.37
2	0.412	35.6	
1	0.492	21.9	
0.5	0.543	13.8	
0.25	0.593	5.9	

Absorbance of control = 0.63
IC_{50} value was calculated by using linear regressive equation.

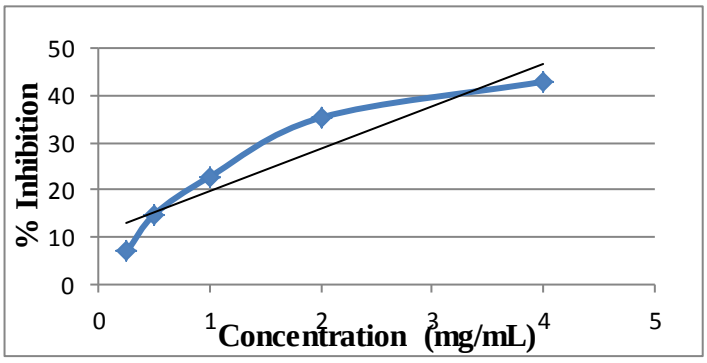

Figure 5. % Inhibition of Different Concentration of Sample

According to table (5) and (6) , IC_{50} value of the standard ascorbic acid was 41.9 µg/mL and ethanol extract of sample was 4.37 mg/mL. The antioxidant activity of the sample was found to be lower than that of standard ascorbic acid.

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4 DISCUSSION AND CONCLUSION

In the present work, the morphological, phytochemical, elemental analysis and antioxidant characters of *Carica papaya* L. were presented. In morphological studies, *Carica papaya* L. was perennial herbs, trunk soft. The stems are unbranched, cylindrical. The leaves were simple, palmately lobed, spiral. These characters are agreed with those mentioned by Dassanayake 1987.

Inflorescences are axillary, much branched cyme with many flowers. Staminate flowers are pale yellow, unisexual, actinomorphic. Pistillate flowers are whitish to yellow flowers, unisexual, actinomorphic. These characters are similar to those given by Lawrence 1965.

Ovary is superior, ellipsoid, unilocular, with many ovules in the locules, parietal placentation. Style selender and stigma are five fan-shaped .These characters are agreed with those given by Dassanayake 1987.

According to the results of phytochemical studies, the leaves of *Carica papaya* L. showed the presence of a wide of secondary metabolites such as alkaloid, glycoside, flavonoid, phenol, polyphenol, saponin, tannin and steroid. Lipophenol and reducing sugar are absent.

From EDXRF data, magnesium, phosphorus, sulfur, potassium, calcium, titanium, manganese, iron, zinc and strontium. Major constituents such as potassium, magnesium, calcium, sulfur and phosphorus were found and minor constituents such as iron, manganese, strontium, titanium and zinc were also found in the male papaya leaves. The amount of potassium, magnesium and calcium were higher than other elements.

Antioxidant activity of ethanol extract of male papaya leaves are investigated by DPPH free radical scavenging method. IC_{50} value of ethanol extract were 4.37 mg/mL.

In Myanmar, a large number of medicinal plants are found as natural resources. Local people are identifying plants based mostly on morphological characters, but they can't be able to identify the dry parts of the medicinal plants.

In conclusion, tannin, phenol, polyphenol and flavonoid which are found in papaya are shown to possess anticancer properties. The body needs a delicate balance of potassium to help the heart and other muscles work property. Calcium support to built and maintain strong bones and protecting against cancer, diabetes and high blood pressure. Antioxidant activity of male papaya leaves is lower than standard ascorbic acid. The male papaya leaves showed antioxidant activity in some extent. Therefore, the male papaya leaves are good for health. The consuming of foods rich in antioxidant may be good for our health and may also help to lower our risk of infection and some forms of cancer. The role of this plant could be assumed to be important in medicinal purposes.

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