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SOME BENEFICIAL PROPERTIES OF CARICA PAPAYA LINN. LEAVES AND HOW THEY VARY ACCORDING TO SEASONS AND SITE OF COLLECTION

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ABSTRACT:

Background: *Carica papaya* Linn, belonging to the family Caricaceae, and it's an important medicinal herb. Different morphological parts of the plant are traditionally used for prevention and treatment of various diseases. However, the medicinal values of herbs are affected by season and site of collection.

Aim: This study evaluated how geographical location and seasons of harvest affect the medicinal properties of *Carica papaya* leaf.

Methods: Thirty *C. papaya* leaf samples were collected from three collection sites in five local government areas of Kaduna state, Nigeria, during rainy and dry seasons. Antioxidant activity of the ethanol extracts of the various samples was determined using DPPH free radical scavenging method, while the elemental analysis was conducted using atomic absorption spectroscopy.

Results: Samples were found to be highly potent in scavenging DPPH free radical with significant ($P < .05$) difference observed between the EC_{50} (efficient concentration at 50 %) of the various samples and ascorbic acid (positive control). The antioxidant activities of the plant were found to be higher in dry season compared to rainy season. Samples were found to be rich in iron, manganese and zinc and were within the FAO/WHO official limits.

Conclusion: *C. papaya* leaf when collected during dry season has higher concentrations of Iron, Manganese and Zinc and also better antioxidant activity than those collected during rainy season.

Keywords: Antioxidant, *Carica papaya*, Essential element, Location, Season

1.0 INTRODUCTION

Carica papaya Linnaeus, pawpaw or papaya in English, belongs to the family of Caricaceae. It is also known as Gwanda in Hausa, okwulu ezi in Igbo, and ibepe in Yoruba [1]. Traditionally, decoction of the different parts of *C. papaya* have been used singly or in combinations for the treatment of malaria, typhoid fever and jaundice [2]. Studies have shown that the ethanol and aqueous extract of the leaf, raw fruit, ripe fruit and dried seed of *C. papaya* have hepatoprotective, diuretic, anti-inflammatory, antihypertensive, antifungal, anthelmintic, anti-sickling, immunomodulatory and antiviral activities [3,4]. Seasons and sites of collection have also been reported to affect the quality of herbal medicines [5, 6 & 7]. Therefore, medicinal herbs are normally harvested in a certain season for their medicinal values. Hence, the need to study the pattern of antioxidant activity and essential elements of *C. papaya* leaf according to location and season of harvest.

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2.0 METHODOLOGY

2.1 Collection, Identification and Preparation of Samples

C. papaya Linn. Leaf samples were collected in February (dry season) and August (rainy season), from Giwa, Makarfi, Soba, Sabon Gari and Zaria Local Government Areas of Kaduna State, Nigeria. The samples were identified and authenticated at the Department of Botany, Faculty of life Sciences, Ahmadu Bello University, Zaria, Nigeria and a voucher specimen number of V/No. A.B.U. 12063 was assigned. The leaf samples were washed over running tap water, shade dried, coarsely size reduced and stored in airtight labelled glass jars containers. They were then coded as S1a-S15a and S1b-S15b for samples collected in rainy and dry seasons respectively.

2.2 Sample Extraction

The leaf samples of the *C. papaya* were extracted with ethanol using cold maceration. A quantities (4.0 g) of each sample were mixed with 100 ml of ethanol in a glass-stoppered conical flasks. The mixtures were then vigorously shaken and allowed to stand for 24 hours. They were filtered and transferred to tarred flat-bottomed dishes and evaporated to dryness in a water bath. The extracts obtained were used for the determination of antioxidant activities and essential elements of the *C. papaya* leaf.

2.3 Antioxidant activity of *C. papaya* leaf samples

The antioxidant activity was conducted using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method [7].

2.3.1 Samples Preparation

DPPH solution (0.1 mg/ml) was prepared by dissolving DPPH powder (10 mg) in 100 ml of methanol. Quantities (10 mg) of the extracts were dissolved in methanol (20 ml) to produce 0.5 mg/ml solutions. Working concentrations (0.5, 0.25, 0.125, 0.0625 0.03125 mg/ml) were then prepared using serial dilutions. Ascorbic acid solution in methanol (positive control) was also prepared using the same procedure.

2.3.2 Antioxidant activities studies of *C. papaya* ethanol leaf extracts

Aliquots (1.0 ml) of the prepared working solutions were added to 1.0 ml of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution. They were vortex mixed and placed in dark room for 30 minutes and their absorbance were measured at 517 nm. The same procedure was repeated for the positive control solution. The percentage antioxidant activities were calculated using the formula below:

$$DPPH\ radical\ scavenging\ activity\ \% = \frac{ABS(DPPH) - ABS(sample)}{ABS\ (DPPH)}\ x\ 100$$

Where, ABS (DPPH) = absorbance of DPPH in methanol
ABS (sample) = absorbance of DPPH in methanol + sample extract

Percentage antioxidant activity values were then plotted against the concentrations and the efficient concentration at 50 % (EC_{50}) of various samples were extrapolated.

2.4 Elemental Analysis of *C. papaya* leaf samples

This was carried out using the procedure of the World Health Organization [8];

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2.4.1 Sample preparation

Quantities (500 mg) of the *Carica papaya* leaf samples were transferred into beakers containing 20 ml concentrated HNO_3. The mixtures were gently boiled at 100 ˚C for 30 minutes to oxidize all easily oxidizing matter and then cooled. 10 ml of 70 % $HClO_4$ was then added and heated until clear solutions were obtained. The solutions were cooled, filtered through Whatman filter papers and made up to 50 ml with deionized water.

2.4.2 Sample Analysis

The digested samples were analysed for the presence of iron, manganese and zinc with atomic absorption spectrophotometer (AAS) using concentrated HNO_3 and 70 % $HClO_4$ as a blank solution. All readings were taken in triplicates and expressed as mean ± SD. Concentrations (µg/ mL) of the analytes were determined using the formula:

$$\text{Element } (\mu g/\text{ ml}) = \frac{(C)(V)(d.f)}{w}$$

Where, C = concentration of the element in the sample solution in µg/ml
V = volume of the undiluted sample solution in ml
d.f = dilution factor
W = sample weight in gram

Data Analysis

Data were analysed using one-way analysis of variance (ANOVA) followed by Turkey HSD post hoc test for multiple comparisons at 95 % confidence interval using IBM SPSS statistics 20.

3.0 RESULTS

C. papaya Leaf Efficient Concentration at 50% (EC_{50})

In both seasons, the *C. papaya* leaf extracts were found to be highly potent in scavenging the DPPH free radical than vitamin-C, as there EC50 were higher than 0.010 mg/ml recorded against vitamin C used as control (Table 1).

Table 1: Efficient Concentration at 50% (EC_{50}) of *C. papaya* Leaf Samples

Mean Efficient Concentration at 50% (mg/ml) ±SD			
Rainy season	**EC_{50}±SD**	**Dry season**	**EC_{50} ±SD**
S1a	0.070* ±0.001	S1b	0.011 ±0.001
S2a	0.088* ±0.001	S2b	0.068* ±0.001
S3a	0.010 ±0.001	S3b	0.043* ±0.001
S4a	0.031* ±0.0001	S4b	0.130* ±0.001
S5a	0.147* ±0.001	S5b	0.102* ±0.001
S6a	0.100* ±0.001	S6b	0.032* ±0.001
S7a	0.089* ±0.001	S7b	0.081* ±0.001
S8a	0.230* ±0.001	S8b	0.292* ±0.001
S9a	0.111* ±0.001	S9b	0.079* ±0.0001
S10a	0.027* ±0.001	S10b	0.081* ±0.001
S11a	0.127* ±0.001	S11b	0.033* ±0.001
S12a	0.158* ±0.001	S12b	0.022* ±0.001
S13a	0.061* ±0.001	S13b	0.010 ±0.001
S14a	0.107* ±0.002	S14b	0.029* ±0.001
S15a	0.224* ±0.001	S15b	0.058* ±0.001

*Significant difference from the control (Vitamin-C EC_{50} 0.010 mg/ml) at $P < .05$

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Assessment of Metallic Constituent of *C. papaya* Leaf Extract

The *C. papaya* leaf extracts were found to be rich in Iron as shown in Table 2 below. In both seasons, the iron concentration was found to be higher than 50 µg/g. However, the iron concentration was higher during the dry season compared to rainy season. Also, sufficient concentration of manganese higher than the FAO/WHO limit of 0.2 µg/g were recorded in all the extract for both dry and rainy seasons (Table 3).

Table 2: Concentrations of Iron in *C. papaya* leaf samples

Mean Iron Concentration (µg/g) ± SD			
Rainy season	**Concentration ± SD**	**Dry season**	**Concentration ± SD**
S1a	127.23*±1.11	S1b	352.43*±0.12
S2a	162.77*±0.05	S2b	423.92*±0.49
S3a	285.50±0.79	S3b	562.58*±0.52
S4a	135.92*±0.18	S4b	240.93*±0.11
S5a	84.78*±0.49	S5b	75.04*±0.11
S6a	104.07*±0.56	S6b	133.17*±0.71
S7a	66.51*±0.27	S7b	241.66*±0.38
S8a	148.39*±0.46	S8b	123.28*±0.27
S9a	235.44*±0.51	S9b	347.34*±0.42
S10a	98.51*±0.31	S10b	239.40*±0.28
S11a	132.27*±0.31	S11b	275.59*±0.46
S12a	103.60*±0.46	S12b	221.61*±0.36
S13a	105.35*±0.42	S13b	207.09*±0.56
S14a	62.79*±0.45	S14b	143.04*±0.62
S15a	128.16*±0.91	S15b	85.80*±0.94

*Significant difference from the control (S3a) at $P < .05$.

Table 3: Concentrations of manganese in *C. papaya* leaf samples

Mean Manganese Concentration (µg/g) ± SD			
Rainy season	**Concentration ± SD**	**Dry season**	**Concentration ± SD**
S1a	16.45*±0.06	S1b	19.47*±0.43
S2a	21.57*±0.23	S2b	24.34*±0.21
S3a	26.72*±0.11	S3b	28.27*±0.09
S4a	23.34*±0.25	S4b	24.32*±0.31
S5a	14.42*±0.35	S5b	12.14*±0.16
S6a	16.67*±0.33	S6b	21.25*±0.19
S7a	15.42*±0.42	S7b	19.44*±0.16
S8a	18.41*±0.17	S8b	22.46*±0.12
S9a	25.61*±0.15	S9b	24.37*±0.44
S10a	21.54*±0.27	S10b	21.35*±0.13
S11a	19.35*±0.02	S11b	24.61*±0.10
S12a	23.41*±0.16	S12b	28.28*±0.16
S13a	21.16*±0.24	S13b	23.45*±0.12
S14a	16.18*±0.06	S14b	19.30*±0.16
S15a	14.88*±0.10	S15b	21.41*±0.17

*Significant difference from the FAO/WHO limit (0.2 µg/g) at $P < .05$.

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Table 4 showed that the ethanol extracts of *C. papaya* leaf was also very rich in Zinc concentration.

Table 4: Concentrations of Zinc in *C. papaya* Leaf Samples

Mean Zinc Concentration (mg/kg) ± SD			
Rainy season	**Concentration ± SD**	**Dry season**	**Concentration ± SD**
S1a	1.13*±0.11	S1b	18.35*±0.12
S2a	6.33*±0.12	S2b	26.24*±0.20
S3a	19.43±0.06	S3b	11.39*±0.14
S4a	23.76*±0.05	S4b	32.78*±0.09
S5a	14.26*±0.16	S5b	23.49*±0.03
S6a	12.24*±0.07	S6b	5.78*±0.06
S7a	9.28*±0.05	S7b	26.47*±0.14
S8a	16.14*±0.04	S8b	21.58*±0.31
S9a	14.17*±0.04	S9b	3.42*±0.02
S10a	31.22*±0.11	S10b	34.65*±0.03
S11a	18.24*±0.02	S11b	27.36*±0.07
S12a	15.18*±0.03	S12b	0.63*±0.03
S13a	6.45*±0.03	S13b	13.68*±0.25
S14a	27.32*±0.03	S14b	28.66*±0.24
S15a	14.07*±0.01	S15b	20.70*±0.15

*Significant difference from the control (S3a) at $P < .05$.

4.0 DISCUSSION

4.1 Antioxidant Activities of *C. papaya* Leaf Samples

Significant ($P < 0.05$) difference was observed between the EC_{50} DPPH scavenging activities of the samples (*Tab. 1*). Potency in the antioxidant activity was generally observed to be higher in samples collected during dry season as compared to those collected during rainy season. Sample S13b (sample from Zaria) was found to be the most potent having the same EC_{50} value with the positive control while the least potent was sample S8b from Soba during dry season. High antioxidant activity was observed in samples collected during the dry season compared to those collected in raining season. This could be attributable to the high concentrations of secondary metabolites during this period [9, 10]. The high antioxidant activity of *C. papaya* leaf further validates it uses as antioxidant and anti-tumour [11, 12].

4.2 Essential Elements in *Carica papaya* Leaf Samples

The *C. papaya* leaf samples were found to be rich in Fe (*Tab. 2*) with significant ($P < .05$) difference according to seasons and sites of sample collections. The highest concentration (285.50 mg/kg) was found in sample collected from Giwa during the dry season (S3b) while the lowest concentration (62.79 mg/kg) was recorded in sample collected from Zaria during the rainy season (S14a). Iron is an essential element required in wide ranges of metabolic processes such as oxygen transport, synthesis of deoxyribonucleic acid (DNA)

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and electron transport [13]. *C. papaya* leaves, being rich in iron, could serve as a nutritional supplement for iron. However, Fe toxicity has been associated to excessive accumulation of iron in the liver, pancreas, heart, lungs and other tissues leads to a toxicological condition called haemochromatosis [14]. Official limit for iron in herbs has not been established, but recommended daily intake of 8 mg/kg and 18 mg/kg for adult male and female respectively have been reported in the literature [15].

The concentrations of manganese (Mn) in the *C. papaya* leaf samples (*Tab. 3*) were found to be significantly ($P < .05$) different from each other based on seasons and sites of samples collection. The highest concentration (28.28 mg/kg) was found in sample from Sabon Gari during dry season (S12b) while the least concentration (12.42 mg/kg) was recorded in sample S5b collected from Makarfi during dry season. Mn is an essential trace element that plays vital roles in the formation of bone, proper function of several metalloenzymes such arginase, glutamine synthetase, phosphoenolpyruvate decarboxylase, and manganese superoxide dismutase [16, 17]. Mn bio-accumulates to toxic level leading to disruption of homeostasis and consequently resulting to fragile bone, kidney stone, hepatic cirrhosis, dystonia and Parkinsonism-like symptoms especially in the elderly with renal diseases [18, 19].

Significant ($P < .05$) difference in zinc concentrations (*Tab. 4*) was observed in the various samples of *C. papaya* leaf. The highest concentration (34.65 mg/kg) was found in sample collected from Soba during the dry season (S10b) while the lowest concentration (0.63 mg/kg) was recorded in sample S12b collected from Sabon Gari during the dry season. Zn is an essential component of a number of enzymes that participates in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids as well as its role in immune system, with major function in cellular and humoral immunity [20]. Severe Zn deficiency in humans are associated with growth retardation, delayed sexual and bone maturation, skin lesions, diarrhoea, alopecia, impaired appetite and increased susceptibility to infections as a result of low immune system [21, 22].

5.0 CONCLUSION

The antioxidant potency of *C. papaya* leaf and the levels of essential metals were found to be significantly ($P < .05$) affected by season and geographical location. Antioxidant potency and amounts of essential metals of *C. papaya* leaf were found to be high during dry season.

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Conflict of interest
We declared that, we have no conflict of interest.

Certification: This research work has not been published or sent simultaneously to other journals for publication.

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