

Quantitative Phytochemical Screening of Male and Female Tree Leaves Of *Carica Papaya* Linn.

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ABSTRACT: The purpose of the present study is to quantify bioactive compounds including alkaloids, phenols, flavonoids and saponins in aqueous and ethanolic extracts of male and female *Carica papaya* Linn tree leaves separately. Phenolic contents in both aqueous and ethanolic extracts of male tree leaves were found to be significantly different as compared to female (p-value <0.01). Alkaloids were found abundantly in both aqueous and ethanolic extracts of female tree leaf (p-value <0.01). Flavonoids were found with no significant difference, in aqueous extracts (p-value =0.01) and in ethanolic extracts (p-value =0.04) using independent t-test. Saponins were also significantly different in both male and female aqueous and ethanolic leaf extracts (p-value <0.01). The results reflected that aqueous extracts from female plant is richer in alkaloids while male leaves are richer in phenols. Significant difference in phenolic contents of male and female tree leaves not only in aqueous but also in ethanolic extracts was observed. Saponin contents determined indicated significant difference in between the two types of tree leaves. The current research showed variations in phyto-constituents in female and male tree leaves of *Carica papaya* L. This data will be utilized for further evaluation of the plant which can not only be useful in distinguishing phytochemicals of *Carica papaya* L. male and female tree leaves but also helps in improving quality standards.

Keywords- Alkaloids, phenolic contents, flavonoids, saponins, quality standard

I. INTRODUCTION

Carica papaya L. is the member of Caricaceae family of plant Kingdom. The plant is named as Papaya in English, Papeeta in Urdu and Erandakarkati in Sanskrit^[1]. Since sixteenth century, the plant has been cultivated in United States of America and tropical region of Subcontinent^[2]. In Punjab and Sindh Province of Pakistan, *Carica papaya* L. is also extensively cultivated^[3]. The plant is identified by its soft unbranched stem. The stem apex secretes milky latex. The plant growth is rapid and attain 20m maximum height^[4]. Traditionally, various extracts of *Carica papaya* L. tree leaves are consumed in various ailments including dengue hemorrhagic fever^[5]. Leaves of *Carica papaya* L. showed beneficial effects. In Asia, leaves of the plant are widely utilized as medicine^{[6]-[9]}. Phytoconstituents of leaf have showed hepatoprotective, hypoglycemic, anti-fertility, immunomodulatory, wound healing as well as antiviral properties^{[10]-[12]}.

II. MATERIALS AND METHOD

2.1 Plant collection

Tall variety of *Carica papaya* L. tree was cultivated in Memon Goath, Malir Karachi in December, 2016. The plants were cultivated at 22% to 60% humidity with temperature range of 20-35°C. The plants were also not pesticised. Plant leaves were taxonomically authenticated by Dr. Shahnaz Dewar, Professor at Department of Botany, Karachi University, Pakistan. Sample voucher specimen No.107 and 108 was registered at Department of Pharmacognosy, University of Karachi, Pakistan. After washing, leaf sample were cut and only lamina part was separated and grounded into powder mechanically and kept separately in air tight jar for future usage^[13].

2.2 Preparation of Aqueous and Ethanolic extract

Female and male tree leaves (500g each) of *Carica papaya* L. were macerated in 1000 ml of distilled water and ethanol separately with proper labelling and kept for 2 weeks in separate wide mouth jars and shaken 10-15 times daily for 10 mins. Leaves were extracted separately with distilled water and ethanol for wide-ranging extraction. The extracts were then filtered by using Whatman filter paper No. 42 and kept at 4°C in air tight container. The solvent filtrates were concentrated in vacuum by Marshall Scientific TM Büchi Rotavapor® R-200 and freeze dried by using KESU-4F® Union Microwave freeze dryer (Zhengzhou) Co., Ltd. Dried

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extracts were stored with proper labelling in separate containers at temperature range of 4° to 8°C for their future use^[14].

2.3 Total phenolic contents determination

The total phenolic contents in both aqueous extract was calculated by Folin-Ciocalteu reagent technique with slight modifications in protocol. 2.5ml Folin-Ciocalteu reagent (10%), 2ml Na₂CO₃ (2%) were mixed to 1ml of plant extract. The resulting mixture was incubated for 15 minutes at ambient temperature. At 765nm, the absorbance was measured. As standard, Gallic acid (1mg/ml) was used. All the tests were performed thrice. The results were determined by the standard curve and expressed as Gallic acid equivalent (concentration as mg/ml of compound extracted)^[15].

2.4 Alkaloid determination

In a beaker of 250ml, 5g sample was weighed and 200ml acetic acid (10%) in ethanol was mixed, shielded and allowed to stand it for the next 4 hours. This material was filtered and concentrated on a water bath. NaOH (1N) was added into this extract dropwise till precipitation occurs. The overall solution was allowed to settle, precipitates were separated and washed using NH₄OH (dil.) and filtered again. Alkaloid was obtained as residue, dried and finally weighed^[16].

2.5 Flavonoid determination

For flavonoid determination, 10g sample was extracted with 80% aqueous methanol (100 ml) repeatedly at ambient temperature. The obtained solution was filtered using Whatman filter paper No 42. Filtrate was shifted in crucible and evaporated over a water bath till it dried and then finally weighed^[17].

2.6 Saponins determination

20 g of each grounded sample and 20% aqueous ethanol (100 cm³) were added into a conical flask. The combination was heated for 4 hrs. With continuous stirring over a water bath at about 55°C. Filtrate was separated and the residue were re-extracted with another 200 ml solvent system. Both extracts were condensed to 40 ml at 90°C over water bath. The concentrate was shifted into a separating funnel (250 ml) and shaken strongly after addition of 20 ml diethyl ether. The ether layer was discarded and only aqueous layer was separated. The process of purification was repeated again. Now n-butanol (60 ml) was added. The mutual n-butanol extracts were washed with 5% sodium chloride solution (10 ml) twice. In a water bath, the residual solution was heated. After evaporating, it was dried in the oven to a constant weight, the saponin content were calculated^[16].

III. RESULTS AND DISCUSSION

Quantitative phytochemistry of male and female tree leaves showed significant difference in phytochemicals. Total phenolic contents, alkaloid contents, flavonoids and saponins from male and female tree leaf in triplicates in both water and ethanol were evaluated. Each value is the average of three analyses (Mean) ± standard deviation (SD).

3.1 Phenolic Content of Extract (In %)

Carica papaya Linn leaves provides wide variety of phyto-constituents for various herbal formulation. The plant is the prime source of phytochemicals, essential vitamins, minerals and other important substances^[18]. Phenolic contents were found to be 38.66 % and 54 % in female and male aqueous leaf extracts respectively while total phenolic contents in ethanolic extracts of female and male were found to be 17.33 % and 28.66 % respectively. Phenolic contents in both aqueous and ethanolic extracts of male tree leaves were found to be significantly different as compared to female (p-value <0.01).

TABLE 1: Phenolic Content of Extract

Extract	Absorbance at 765nm	% contents (GAE)	Mean (%)
ALEM	0.26	52%	54 ± 1.63
	0.28	56%	
	0.27	54%	
ALEF	0.19	38%	38.66 ± 2.49
	0.21	42%	
	0.18	36%	
p-value	<0.01*		

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ELEM	0.15	30%	28.66 ± 0.94
	0.14	28%	
	0.14	28%	
ELEF	0.11	22%	17.33 ± 3.39
	0.7	14%	
	0.8	16%	
p-value	<0.01*		

ALEM, Aqueous Leaf Extract Male; ALEF, Aqueous Leaf Extract Female; ELEM, Ethanolic Leaf Extract Male; ELEF, Ethanolic Leaf Extract Female) Note: Each value is the average of three analyses (Mean) ± standard deviation (SD)

TABLE 2: Concentration Vs Absorbance of Gallic acid

No.	Concentration (mg/ml)	Absorbance
1	1	0.17
2	1.5	0.27
3	2	0.43
4	2.5	0.61
5	3	0.72
6	3.5	0.82
7	4	0.95
8	4.5	1.16

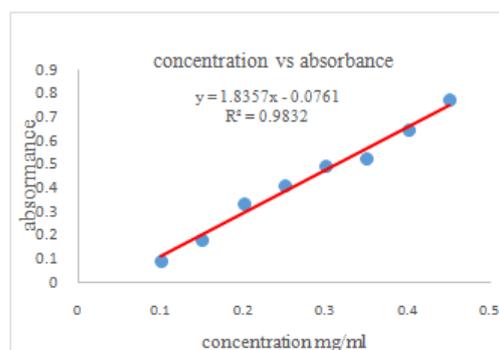


FIG 1. Calibration curve of Gallic acid

3.2 Alkaloid Contents

Alkaloids were found to be 22.43% and 39.2% in male and female aqueous leaf extracts respectively while in ethanolic extracts of male and female it was found to be 21.46% and 34.8 % respectively. Alkaloids were found abundantly in both aqueous and ethanolic extracts of female tree leaf (p-value <0.01) [1].

TABLE 3: Alkaloid Contents

Male plant leaves	Weight of residue G	Weight of plant sample taken g	% mean of alkaloids
Aqueous extract	1.12	5	22.4 ± 0.588
Ethanolic extract	1.07	5	21.46 ± 0.736
Female plant leaves	Weight of residue	Weight of the plant sample taken g	% mean of alkaloids
Aqueous extract	1.96	5	39.2 ± 1.177
Ethanolic extract	1.74	5	34.8 ± 0.748
Aqueous p-value <0.01* Ethanolic p-value <0.01* using independent t-test			

3.3 Flavonoid Contents

Flavonoids were found to be 12.16% and 13.2 % in male and female aqueous leaf extracts respectively while in ethanolic extracts of male and female it was found to be 70.66% and 72.96 % respectively. Flavonoids were found with no significant difference.

Male plant leaves	Weight of residue g	Weight of plant sample taken g	% mean of flavonoids
Aqueous extract	1.21	10	12.16 ± 0.169
Ethanolic extract	7.09	10	70.66 ± 1.24
Female plant leaves	Weight of residue	Weight of the plant sample taken g	% mean of flavonoids
Aqueous extract	1.32	10	13.2 ± 0.372
Ethanolic extract	7.29	10	72.96 ± 0.543
Aqueous p-value =0.01* Ethanolic p-value =0.04* using independent t-test			

TABLE 4: Flavonoid Contents

3.4 Saponin Contents

Saponins were found to be 28.45% and 33.93% in male and female aqueous leaf extracts respectively while in ethanolic extracts of male and female it was found to be 31.3% and 34.93% respectively. Saponin contents determined indicated significant difference in between the two types of tree leaves

Table 5: Saponin Contents

Male plant leaves	Weight of residue g	Weight of plant sample taken g	% mean of saponins
Aqueous extract	5.69	20	28.45± 0.348
Ethanolic extract	6.26	20	31.3± 0.147
Female plant leaves	Weight of residue	Weight of the plant sample taken g	% mean of saponins
Aqueous extract	6.78	20	33.93± 0.714
Ethanolic extract	6.98	20	34.93 ±0.510

Aqueous p-value <0.01* Ethanolic p-value <0.01* using independent t-test

FTIR analysis evaluated functional groups separately for both types of leaf extracts reflected significant difference in both plants leaves constituents. More functional group peaks are observed in aqueous female tree leaves extracts^[2]. The results revealed that aqueous extracts from female plant is richer in alkaloids and phenols as compared to male. Significant difference in the values of phenolic contents of male and female leaves was found not only in aqueous but also in ethanolic extracts.

IV. CONCLUSION

Carica papaya Linn leaves is a hub of phyto-constituents. The current research showed variations in phyto-constituents in female and male tree leaves of *Carica papaya* L. Significant difference in phytoconstituents was observed in between the aqueous and ethanolic extracts of *Carica papaya* L. male and female tree leaves. This data will be utilized for further evaluation of the plant and helps in improving quality standards as the plant has got importance in disease like dengue.

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