Development of quality standard and phytochemical analysis of *Carica papaya* Linn leaves

Syed Zohaib Hussain[1]*, Nighat Razvi[2], Syed Imran Ali[3] and Syed Mohammad Farid Hasan[4]
[1]College of Pharmacy, Liaquat University of Medical and Health Sciences, Jamshoro, Pakistan
[2]Faculty of Pharmacy, Nazeer Hussain University, Karachi, Pakistan
[3]Faculty of Pharmacy, Ziauddin University, Karachi, Pakistan
[4]Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi, Pakistan

Abstract: *Carica papaya* Linn is the member of Caricaceae family of Kingdom Plantae. The study was executed for the development of qualitative standards of male and female leaves of the plant. The study included evaluation of macroscopial, physico-chemical and preliminary phytochemical parameters to authorize the purity and authenticity of leaf of *Carica papaya* Linn based on guidelines provided by WHO. Qualitative phytochemical screening of extracts revealed the existence of alkaloids, flavonoids, tannins, phenolic compounds, glycosides including cardiac glycosides, proteins and carbohydrate in different extracts of *Carica papaya* Linn which are majorly rich in female leaves as compared to male. Mean ash values, acid insoluble ash, water soluble ash, foaming index, swelling index and moisture contents were also evaluated which are more or less similar. FTIR profile of the samples were also generated that confirmed distinct peak values with respective functional groups exhibited by *Carica papaya* male and female plant. The current research reflected that female and male plant showed variations in phyto-constituents. This data will be utilized for additional Pharmacological and Instrumental evaluation of the plant which can not only be beneficial in discriminating and refining the type as well as nature of various phytochemicals present in *Carica papaya* male and female leaves but also establish the quality standards for future researches.

Keywords: *Carica papaya*, Caricaceae, phytochemical screening, FTIR, functional groups, WHO.

INTRODUCTION

Carica papaya Linn is the member of family Caricaceae. The plant is known as Papaya in English, Papeeta in Urdu. In Sanskrit, it is named Erandakarkati (Anjum *et al.,* 2013*)*.The plant is native to tropical region of United States of America (Milind and Gurditta, 2011) and was cultivated in Subcontinent in the near 16th century. In Pakistan, the plant is extensively cultivated in Sindh and some regions of Punjab. *Carica papaya* plant is renowned by its soft unbranched shoot that produces chalky or creamy latex not only at the apex of long stalked leaves, but also as a constituent of fruit usually at unripe stage. The plant shows rapid growth pattern and can achieve up to the height of 20 m (Owoyele *et al*., 2008) .Traditionally, the leaves are used as medicine for management of pyrexia, vitamin deficiency diseases like beriberi, in asthmatic attacks, in colic pain, against malaria in Asia (Nguyen *et al*., 2016). Leaves in aqueous form have been recommended for treatment of conditions like dengue hemorrhagic disease and jaundice (Ahmad *et al*., 2011). The plant leaves also shows antiviral potential and immunomodulatory effects (Yogiraj *et al.,* 2014).

Papaya plant has momentous health profile chiefly among consumers in Asia (Anuar *et al*., 2008; Lim, 2012; Otsuki *et al*., 2010). Papaya leaf is the richest source of alkaloids including carpain and pseudocarpain. Enzymatic potential includes papain, chymopapain and cystatin. Vitamins and their derivatives including Tocopherol and nicotinic acid. Tannins and saponins are also found (Duke, 2011; Suresh *et al*., 2008). Phenolic acids including caffeic acid, p-coumaric acid and protocatechuic acid as the core phytochemicals of the plant (Canini *et al*., 2007). Flavonoids including kaempferol and myricetin are rich in leaves (Anjum *et al.,* 2013*)*. Young leaf and fruit of the *Carica papaya* Linn exhibits carotenoids specifically lycopene, β- carotene, and glycosidic anthraquinones (Baqi *et al*., 2009). *Carica papaya* leaves possess medicinal aspects like anti-inflammatory effects, hypoglycemic activity (Adeneyea *et al*., 2009), anti-fertility, abortifacient (Pokharkar *et al*., 2010), hepatoprotective and wound healing properties Antitumor activities are also reported .*Carica papaya* leaves are being used in numerous traditional formulations so standardization of various parameters is obligatory.

MATERIALS AND METHOD

Plant collection

Carica papaya Linn tall plant variety was cultivated in the month of December 2016, in Darshan Channa (Memon Goath) Khokrapar Malir, Karachi, Pakistan. The plant was grown in the temperature range of 20-35°C with humidity ranging from 22% to 60% during the whole development period. Plants were not pesticised throughout cultivation session. Leaves of *Carica papaya*

**Corresponding author:* e-mail: zohaib.hussain@lumhs.edu.pk

Linn from both Male and Female plants were collected in the month of May 2017 from the mid half of the plant during morning timings between (09:00 a.m. to 11.00 p.m.) and packed in polyethylene bags during transport. Temperature during collection was 32°C and humidity was 44%. The leaf samples were taxonomically identified and authenticated as tall variety by Professor Dr. Shahnaz Dewar, Department of Botany, University of Karachi, Pakistan. A voucher specimen No.107 and 108 was deposited in Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi, Pakistan.

Samples were washed in Research Lab of Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi to remove dust thrice with distilled water. Distilled water was prepared by using Distillation apparatus (BOECO Germany, Model No. BOE 8707500), Parts of the leaf were separated (Lamina, petiole and mid rib) by using sterile surgical blade purchased from Pin Tech Instruments, Punjab, Pakistan. Only lamina part was used. Leaves of *Carica papaya* were air-dried at room temperature (30±2°C) for two weeks to remove moisture. The plant materials were shade dried until all the water evaporated and plants became fully dried for grinding. Then plant material was grounded well using mechanical blender into coarse powder and placed in air tight jar with proper labeling for future use at ambient temperature to prevent from moisture. Male and female plants powders were separately placed and labeled.

Preparation of extracts

Aqueous extract

The dried grounded leaves of male and female plants were weighed and macerated separately in distilled water i.e. 2000 grams of leaf: 4000 ml of water in separate wide mouth jars. The samples were soaked at 25-30°C for 12 hours with constant shaking after every hour for 10-15mins. The extracts were filtered using Whatman filter paper No 42 and the filtrate was kept in air tight container at 4°C.The solvent filtrates were then concentrated in vacuum using Büchi Rotavapor® R-200 and then freeze dried (Omojasola and Awe, 2004). The dried extract was kept between 4°C and 8°C for their forthcoming use in phytochemical analysis. Percentage yield was also calculated.

Ethanolic and n-hexane extracts

Ethanolic and n-hexane samples of leaves from male and female plants containing 2000 grams of leaf in 4000 ml of ethanol and n-hexane were kept for 2 weeks in separate wide mouth jars with shaking 10-15 times every day for 10 mins. Leaves were extracted separately with ethanol and n-hexane for wide-ranging extraction. The extracts were filtered using Whatman filter paper No 42 and the filtrate was kept in air tight container at 4°C The solvent filtrates were then concentrated in vacuum using Büchi Rotavapor® R-200 and then freeze dried (Omojasola and Awe, 2004). Percentage yields were also calculated.

Physicochemical standardization

Using standard Quality control methods for herbal materials, values were extracted for extraction methods in which 2 to 4 gm of coarse powder sieved using 40 mesh size was dissolved in 100 ml of polar to non-polar solvent separately. Ash values including total ash, acid-insoluble ash and water soluble ash, foaming index, loss on drying, swelling index etc. were determined separately for male and female leaves of *Carica papaya* L. following WHO guidelines (World Health Organization, 2011). All parameters were determined in triplicates.

Determination of ash values

Ash values were calculated by using three methods by which total ash, acid-insoluble ash and water soluble ash was determined (Reddy *et al*., 1999; Kulkarni *et al*., 2008).

Total ash

Air-dried crude drug (2-4g) was weighed in silica dish, incinerated near temperature 4500°C until it was free from carbon completely. In suitable desiccators, residue then allowed to cool for half an hour and weighed quickly. Total ash value (%) was calculated by:

$$\text{Total ash (\%)} \frac{\text{Weight of ash obtained}}{\text{Weight of air dried sample}} \times 100$$

Acid insoluble ash

Ash was boiled with 2M HCl (25 ml) for 5 minutes, the unsolvable material was taken on filter paper (ash less), it was washed with boiling water, burnt, then cooled in desiccators and finally weighed. The value of acid-insoluble ash was evaluated.

Water-soluble ash

With 25 ml of water, the ash was boiled for 5 minutes, insoluble matter was separated on ash less filter paper, washed with boiling water, then ignited for 20 mins, finally cooled using desiccators and then weighed. The % of water-soluble ash was calculated.

Determination of pH 1% solution

1 gm powder drug was accurately weighed and dissolved in 100 ml of accurately measured distilled water, the sample was then filtered and pH was determined by using Table-Top Digital pH meter Labtronics brand Model No. LT-50 pH Meter.

Determination of pH 10% solution

10 gm powder drug was accurately weighed and dissolved in 100 ml of accurately measured distilled water, the sample was then filtered and pH was determined.

Moisture contents

10 g powdered content was placed in pre-weighed watch-glass and then dried at temperature of 100 °C to 105 °C in

an oven over night. Sample contained in watch glass is cooled down at ambient temperature in a desiccator. Final weight was calculated. The loss in weight of the sample is considered as moisture content. Using the formula, moisture content was calculated (Raghuramula *et al*., 2003).

$$\% \text{ Moisture} = \frac{\text{Toal weight - Final weight}}{\text{Weight of sample}} \times 100$$

Determination of swelling index

1gm of *Carica papaya* L. leaf was weighed, that was previously reduced to the essential fineness and transferred into 25ml measuring cylinder with glass stopper.25ml of distilled water was supplemented and shaken thoroughly after every 10 minutes. This shaking was lasted for 1 hour then allowed to stand for 3hours at ambient temperature. Mean value of the separate determinations was then calculated (World Health Organization, 2011).

Determination of foaming index

1g of *Carica papaya* L. leaf was reduced to coarse powder, weighed and transferred into Erlenmeyer flask containing 100ml distilled water which was sustained at modest boiling for half an hour. The material was cooled and then filtered into 100ml Erlenmeyer flask. The decoction was dispensed into 10 test tubes from 1ml to10ml in respective tube with final volume makeup 10ml and stoppered. The tubes were shaken end-to-end motion for 15 to 20 seconds with amplitude of two shakes/second mechanically. All the tubes were allowed to stand in rack for 15 mins and then height of foam were calculated (World Health Organization, 2011).

Powder drug reaction with different reagents

Dried powdered was treated individually with different reagents including Water, base including KOH, NaOH, acids like H_2SO_4, HCl, HNO_3, also chloroform, ferric chloride, ammonia solution, iodine and picric acid were used. The color detected was noted down visually and using microscopy (Sama *et al*., 1994).

Qualitative phytochemical screening

Test for Terpenoids (Salkowski test)

In a test tube, 1 ml of extract was mixed with 1 ml of chloroform and left to evaporate. Then 1 ml of conc.H_2SO_4 was added to the mixture and heated for 2 minutes. Formation of grey color indicated the presence of terpenoids in extracts (Kavit *et al*., 2013).

Test for flavonoids (Alkaline reagent test)

20% NaOH solution was mixed up with extract in the ratio of 1:2. Yellow coloration appeared which transform to colorless upon adding dil. Hydrochloric acid indicated positive test (Ugochukwu *et al.,* 2013).

Test for alkaloids

Add and stirred 5 ml of the 1% aqueous HCl acid with 0.5 g of the extract on steam bath. Treat 1ml filtrate with few drops of dragendorff's reagent. Turbidity appearance was taken as evidence for the alkaloids presence (Adegoke *et al*., 2010).

Test for volatile oils

1 ml ethanol (90%) and 1 ml extract was added in a test tube, add few drops of $FeCl_3$ solution in it. Green coloration indicated volatile oils (Trease and Evans, 1989).

Test for saponins (Frothing test)

In a test tube, half grams of the extract was dissolved in half ml distilled water. Froth persisted upon warming considered as primary indication for Saponins (Adegoke *et al*., 2010). Also Saponins occurrence in extracts was positive if constant and stable foaming appears when extract and distilled water were mixed in the same proportion with vigorous shaking.

Test for cardiac glycosides

0.5 ml of glacial acetic acid along with a drop of $FeCl_3$ solution in a test tube having 1 ml of extract. Brown ring was developed at the interface indicated cardiac glycosides when 0.5ml of conc. H_2SO_4 was added to that mixture (Ugochukwu *et al*., 2013).

Test for coumarins

On filter paper, 5-6 drops of NH_3 solution were dropped. Now, a drop of the extract was added to the filter paper and the paper was observed for fluorescence.

Test for steroids (Liebermann–Burchard reaction)

Add 1ml each of chloroform extract and 1ml conc. H_2SO_4 in a test tube. Appearance of red color in the inferior layer of chloroform dictated presence of steroids in the sample (Kavit *et al*., 2013).

Test for tannins and phenolic compounds

5ml Extract with acetic acid if provides Red colored solution, indicated tannins and phenolic compounds (Boxi *et al.,* 2010).

Test for phlobatannins

Aqueous plant extract when mixed with 1% HCl (aqueous) red precipitate gave evidence of phlobatannins (Edeoga *et al.,* 2005).

Test for anthroquinones - Borntrager's test

10 ml benzene and extract shaken well and filtered. To filtrate, 5 ml of NH_3 solution (10%) was added and stirred. Pink red or violet coloration indicated the free anthroquinones in sample (Sofowara, 1993; Harborne, 1998).

Test for betacyanins

2 ml extract and 1 ml NaOH (2N) was mixed in test tube and heated at 100^{o}C for 5-6 minutes. Yellow coloration reflected betacyanins in sample (Harborne, 1973).

Test for quinones
1 ml extract and 1 ml of H_2SO_4 (conc.) was mixed in the test tube. Red coloration indicated quinones in the sample (Evans, 1996).

Test for acids
1 ml extract and 1 ml of $NaHCO_3$ solution was mixed in attest tube. Effervescence showed the presence of acids in the sample.

Test for Carbohydrates: Molisch test (for Carbohydrates)
2 drops of α napthol solution added in alcohol to which 2–3 ml of aqueous extract was mixed, shake well and add conc. H_2SO_4 in test tube from corners. Violet ring appeared (Boxi *et al*., 2010).

Barfoed's test (for Monosaccharide)
Equal quantity of extract and Barfoed's reagent was mixed. Heated for 2 minutes in water bath and then allowed to cool. Brick red color indicated monosaccharides (Boxi *et al*., 2010).

Iodine test (for Starch)
2ml Iodine solution was mixed with 2ml of Crude extract. Purple or dark blue color indicated the starch.

Test for reducing sugars (Fehling test)
1 ml of both Fehling's A and Fehling's B solutions were added in a test tube and boiled for 1 minute. Equal extract solution was added into it. First yellow ppt. then a brick red ppt. was observed when heated in water bath for 5–10 minutes (Boxi *et al*., 2010).

Test for proteins biuret test
In a test tube, 3 ml extract, 4% NaOH and few drops of $CuSO_4$ (1%) solution was added. Violet or Pink color indicated proteins in the sample (Boxi *et al*., 2010).

Test for amino acids ninhydrin test
0.25% Ninhydrin reagent and extract were added to the test tube, boiled it for few minutes. Blue coloration showed amino acids in the sample (Yasuma and Ichikawa, 1953).

Test for resins - acetone-H_2O test
Acetone and extracts were treated. Add small quantity of water and shaken. Turbidity indicated resins in the sample.

Test for fatty acids
0.5 ml extract was mixed well with ether (5 ml). Mixture was placed on the filter paper and allowed to vaporize and dried. Transparence areas on filter paper indicates fatty acids.

Test for gums and mucilages
5ml extract was slowly mixed with 5ml alcohol (absolute) under continuous stirring. Precipitation indicated gums and mucilages (Whistler and Bemiller, 1993).

Fluorescence analysis
In order to study the fluorescence nature, *Carica papaya* L. leaf powder was separately treated with Water, base including KOH, NaOH, acids including 1N HCl, 1N (half strength) HNO_3, and H_2SO_4 and picric acid, Chloroform, petroleum ether, 5% $FeCl_3$ and NH_3 solution. Fluorescence analysis is one of the identification method because when powder is exposed to UV light at different wave length and day light. The fluorescence characters of *Carica papaya* L. leaf were studied in day light as well as in UV light. Wave length used were 254 and 366 nm (Chase and Pratt, 1949; Kokoski *et al*., 1958; Mukherjee, 2010).

Fourier transform infrared spectrophotometer (FTIR) analysis
FTIR is the most leading tool for identification of characteristic functional groups found in the phytochemicals. *Carica papaya* Linn dried aqueous powder from both the male and female plant extract was taken for active analysis. For FTIR study, dried powdered material was loaded on FTIR spectroscope (Thermo Nicolet Avatar-330 FTIR) having range of Scan from 500 to 4000 cm-1.

RESULTS

Macroscopy of male leaf
Physical analysis of the whole *Carica papaya* L. leaves was conducted using sunlight and simulated source. Gross examination revealed that *Carica papaya* L. leaves were broader with length (9-17cm) and girth (22-36cm). Leaves were present laterally on woody shoot (3 to 6 ft.) with large petiole and their lamina having 6-12 main lobes with reticulate venation. The petioles were dull green colored though leaves were dark green with green midribs, veins and veinlets. The petiole was hollow and firm but soft at node and rigid at base of leaves. Dorsal view of the leaf is given in fig. 1. Organoleptic evaluation of male leaf is given in table 1.

Macroscopy of female leaf
Physical analysis of the whole *Carica papaya* L. leaves was conducted using sunlight and simulated source. Gross examination revealed that *Carica papaya* L. female leaves were broader and larger than male leaves with length (30-40cm) and girth (14-19cm). Leaves were present laterally on woody shoot stem (4 to 7 ft.) with large petiole and their lamina having 7-14 main lobes with reticulate venation. The petioles were slightly yellow green though leaves were green to yellow green with light green midribs, veins and veinlets. The petiole was hollow, long and firm at node and rigid at base of leaves. Dorsal view of the leaf is given in fig. 2. Organoleptic evaluation of female leaf is given in table 1.

Table 1: Organoleptic evaluation

Organoleptic evaluation of male leaf		Organoleptic evaluation of Female leaf	
Lamina	Dark green	Lamina	Dull green
Texture	Smooth	Texture	Fine
Nature of powder	Slightly irritating	Nature of powder	less irritating
Taste	Bitter	Taste	Bitter
Odour	Characteristic	Odour	dense

Fig. 1: Dorsal view of the male leaf.

Fig. 2: Dorsal view of the female leaf.

Table 2: Physicochemical characterization

Parameters	Plant leaves	Total ash	Acid insoluble ash	Water soluble ash
Ash values	Male	16.33 ± 0.225	4.70 ± 0.211	6.69 ± 0.101
	Female	18.65 ± 0.321	5.25 ± 0.209	7.95 ± 0.116
Mean pH values		pH 1% solution	pH 10% solution	
	Male	6.88 ± 0.077	5.90 ±0.071	
	Female	6.47±0.055	5.53±0.064	
Loss on drying:		Percentage%		
	Male	6.22 ± 0.023		
	Female	6.51 ± 0.011		
Swelling index		Height		
	Male	0.5cm, Less than 100		
	Female	0.7cm, Less than 100		
Foaming index		Height		
	Male and Female	Less than 100		

Table 3: Percent yield of plant

Extract	Plant	Calculation	% yield
Aqueous	Male	325/2000*100	16.50%
	Female	478/2000*100	23.90%
Ethanol (abs)	Male	296/2000*100	14.80%
	Female	390/2000*100	19.50%
N-hexane	Male	190/2000*100	9.50%
	Female	240/2000*100	12%

Table 4: Powder drug reaction with reagents

Reagents	Color analysis of leaves		Reagents	Color analysis of leaves	
	Male	Female		Male	Female
NaOH(1N)	Green	Green	$FeCl_3$ (5%)	Light brown	Dark brown
H_2SO_4 (conc.)	Dark brown	Dark brown	NH_3 solution	Plate green	Plate green
HCl (conc.)	Blue green	Green	Picric acid	Dull green	Dull green
HNO_3 (conc.)	Reddish brown	Dark brown	Iodine	Brown	Brown
Chloroform	Green	Green	KOH (1N)	Green	Green

Table 5: Fluorescence analysis

Treatment	Observations					
	Day light		UV 254		UV 336	
	Male leaf	Female leaf	Male leaf	Female leaf	Male leaf	Female leaf
Powder	Green	Green	Dark Green	Dark green	Dark Green	Dark Green
Distilled water	Dark Green	Dull Green	Dark Green	Dark Green	Plate Green	Plate Green
NaOH (1N)	Light Green	Dark Green	Dark Green	Dark Green	Bluish green	Green
KOH (1N)	Light Green	Light Green	Brown	Dark Brown	Black Brown	Black Brown
H_2SO_4 (50%)	Brown.	Brown	Dark Brown	Dark Brown	Green	Dark Green
HNO_3 (50%)	Brown	Brown	Black	Bluish Black	Dark Green	Dark Green
HCl (50%)	Plate Green	Plate Green	Brown	Dark Brown	Dark Green	Dark Green
Chloroform	Dark Green	Dark Green	Dull Green	Green	Light Green	Light Green
$FeCl_3$ (5%)	Yellow Green	Yellow	Brown	Brown	Light Brown	Dark Brown
Acetone	Blue Green	Blue Green	Brown	Light Brown	Black	Black
Petroleum ether	Green	Green	Light Brown	Light Brown	Dark Brown	Dark Brown
Picric acid	Plate Green	Plate Green	Green	Green	Dark Green	Dark Green
Ethanol (abs.)	Dark Green	Dark Green	No fluorescence	No fluorescence	Black	Black

Table 6: Qualitative phytochemical screening

Phyto-chemicals	Aqueous leaf extract		Ethanolic leaf extract		N-hexane leaf extract	
	Male	Female	Male	Female	Male	Female
Terpenoids	-	-	++	+++	-	-
Flavonoids	++	+++	-	-	-	-
Alkaloids	++	+++	+	+	-	-
Volatile oils	-	-	++	++	+	+
Saponins	+++	++	++	++	++	++
Cardiac glycosides	++	+++	-	-	-	-
Coumarins	+	-	-	-	-	-
Steroids	-	-	-	-	++	++
Tannins	+	+	++	++	-	-
Phenolic Compounds	+++	+	+	+	+	+
Phlobatannins	++	++	+	+	-	-
Anthroquinones	++	++	-	-	-	-
Betacyanins	+++	+++	-	-	-	-
Quinones	++	++	+	+	-	-
Acids	++	++	++	++	-	-
Carbohydrates	++	+++	-	-	+	+
Monosaccharide	+++	+++	-	-	-	-
Starch	+++	+++	-	-	-	-
Reducing Sugars.	++	++	+	+	-	-
Proteins	++	+++	++	++	-	-
Amino acids	+++	++	++	++	++	++
Resins	+++	++	-	-	-	-
Fatty acids	-	-	-	-	+++	+++
Gums	-	-	+	++	-	-
Mucilages	+	-	+	-	-	-

(Note: +++ strongly present; ++ present; + poorly present; - not detected)

Table 7: Peak values and functional groups

Female leaf aqueous extracts peak values cm^{-1}	Functional groups	I	Male leaf aqueous extracts peak values cm^{-1}	Functional groups	I
1760.73	C=O Aromatic Carbonyl group Stretching	S	--	--	-
--	--	-	1192.74	C–O Stretching	M-S
2937.57	C–H Stretching	S	2880.36	C–H Stretching	S
2365.49	C≡C	S	2361.41	C≡C	S
824.97	=C–H Bending	S	995.73	=C–H Bending	S
--	--	-	3338.02	O–H Acid /Phenol	S
1650.4	C=C	S	--	--	
1527.81	N-H Bending	M-S	1535.98	N-H Bending	M-S

(Note: I; intensity, S; strong, M; medium)

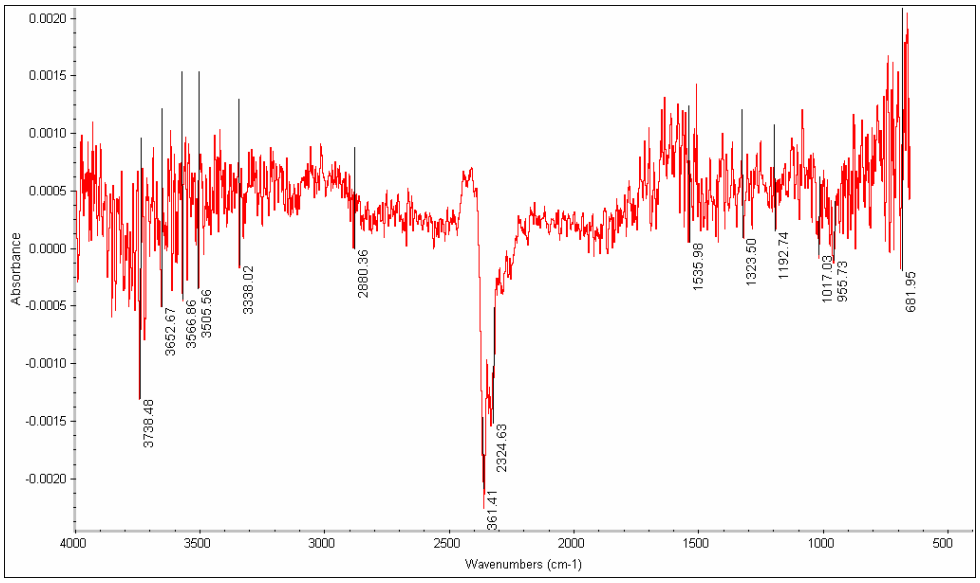

Fig. 3: Interferogram of male aqueous leaf extract

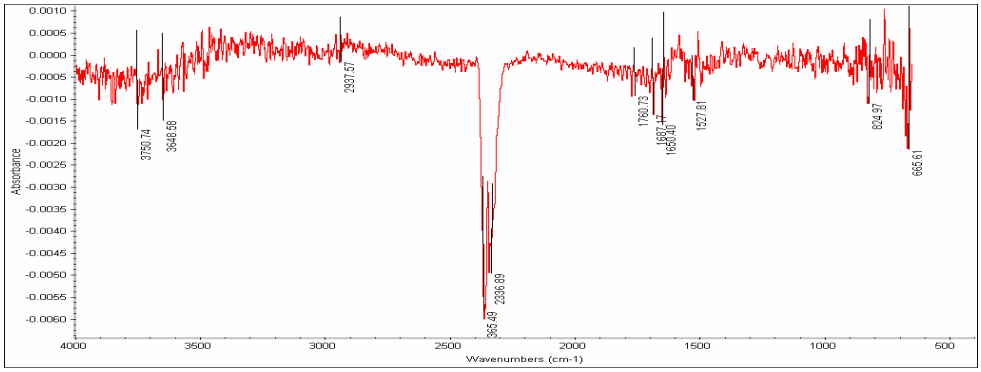

Fig. 4: Interferogram of female leaf aqueous extract

DISCUSSION

Carica papaya Linn leaves are promising in providing wide variety of phyto-constituents for various herbal formulation using in the current era. Previously useful study was conducted for diagnostic setting for authentication and identification in making profile of *Carica papaya* plant (Zunjar, 2011). Development of quality standards of leaves of *Carica papaya* Linn were also studied and established (Anjum *et al*., 2013) to confirm purity and authenticity of leaf of *Carica papaya* L. on the basis of guidelines of WHO but the data was generalized on the leaves of the plant that did not show the difference of male and female leaves. Qualitative phytochemical screening of *Carica papaya* extracts revealed the presence of alkaloids, flavonoids, tannins, phenolic compounds, glycosides including cardiac glycosides, proteins and carbohydrate in different extracts of *Carica papaya* Linn which are similar as previously reported (Anjum *et al*., 2013).This study showed that female leaves are more rich in various compounds as compared to male leaves. Mean ash values, acid insoluble ash, water soluble ash, foaming index, swelling index and moisture contents were also evaluated and provided in table 2. Qualitative standards are established separately for male and female plant leaves in this study. Percentage yield was also evaluated that showed more yield of extract in female as compared to male leaves indicating female leaves as a hub for more yield of extracts in all the three solvents used.

Fourier Transform Infrared Spectrophotometric analysis was also carried out to evaluate various functional groups. The Interferogram revealed significant different in leaves both the plants. Aromatic Carbonyl group is dominating in female leaves while O–H group is dominating in male leaves as indicated by FTIR. On the basis of results obtained in the current study, it can be concluded that the leaf of male plant showed more O-H group indicating presence of more phenolic or acidic contents in male leaves. Significant difference in functional group between the two plants was detected by FTIR that reflected that

female plant is more dominating in phytoconstituents as given in table 6. FTIR Analysis also reported the characteristic functional groups like carboxylic acids, amides, polysaccharides, aldehydes etc. responsible for various medicinal characteristics of the plants.

CONCLUSION

Physicochemical standardization provides same purity and quality of the constituents. Standardization gives assurance of the active principles and marker compounds in a plant material (Garg *et al*., 2010). The present research conducted separately on male and female plant leaves will improve the present established data concerning *Carica papaya* L. leaves to enhance and improve the quality control standards of herbal preparation containing leaves as main ingredients of the formulation against dengue and other diseases reported (Arvind *et al.*, 2013).The current research reflected that female and male plant showed variations in phyto-constituents. This data can not only be beneficial in discriminating and refining the type and nature of various phytochemicals present in *Carica papaya* L. male and female leaves but also establish the quality standards for future researches.

ACKNOWLEDGEMENT

I am very thankful to Dr. Mohtasheem Hasan, Chairman, Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi for his kind cooperation and motivation during my work.

REFERENCES

Adegoke AA, Iberi PA., Akinpelu DA, Aiyegoro OA and Mboto CI (2010). Studies on phytochemical screening and antimicrobial potentials of Phyllanthus amarus against multiple antibiotic resistant bacteria. *Int. J. Appl. Res. Nat. Prod.* **3** (3): 6-12.

Adeneyea AA and Olangunju JA (2009). Pharmacognostic evaluation of *Carica papaya* L. leaves with reference to sexual dimorphism, *Biol and Med*., **1**(1): 1-10.

Ahmad N, Fazal M, Ayaz M., Abbasi BH, Mohammad I and Fazal H. (2011). Dengue fever treatment with *Carica papaya* leaves extracts. *Asian Pac. J. Trop. BioMed*., **1**: 330-333.

Aiyegroro OA and Okoh AI (2010). Preliminary phytochemical screening and in vitro antioxidant activities of aqueous extract of *Helichrysum longifolium* DC. *BMC Compl. and Alt. Med.,* **10**: 21.

Anjum V, Ansari SH, Naquvi KJ, Arora P and Ahmad A (2013). Development of quality standards of *Carica papaya* Linn. leaves, *Sch. Res. Lib.,* **5**(2): 370-376.

Anuar NS, Zahari SS, Taib IA, and Rahman MT (2008). Effect of green and ripe *Carica papaya* epicarp extracts on wound healing and during pregnancy. *Food and Chem. Toxicol.,* **46** (7): 2384-2389.

Arvind G, Bhowmik D, Duraivel S and Harish G (2013). Traditional and medicinal uses of *Carica papaya*, *J. Med. Car. Pap.,* **1(**1): 2320-3862.

Ayoola GA, Coker HAB, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC and Atangbayila TO (2008). Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in South Western Nigeria, *Trop. J. Pharm. Res*, **7**: 1019-1024.

Baqi Y, Atzler K, Kose M, Glanzel M and Muller CE (2009). High-Affinity, Non-Nucleotide-Derived Competitive Antagonists of Platelet P2Y12 Receptors *J. Med. Chem.,* **52**: 3784-3793.

Boham BA and Kocipai AC (1974). Flavonoids and condensed tannins from leaves of *Hawaiian vaccinium* vaticulatum and *V. calycinium*. *Pacific Sci*.,**48**:458-463.

Boxi M, Rajesh Y, Raja Kumar V, Praveen B and Mangamma K (2010). Extraction, phytochemical screening and in-vitro evaluation of anti-oxidant properties of *Commicarpus chinesis* (aqueous leaf extract). *Int. J. Pharm. Biosci*. **1**: 537-547.

Canini, Alesiani A, D'Arcangelo D and Tagliatesta GP (2007). Gas chromatography mass spectrometry analysis of phenolic compounds from *Carica papaya* L. leaf. *J. of Food Compos and Analysis,* **20**(7): 584-590.

Chase JA and Pratt RJ (1949). Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification, *J. American Pharmaceut. Assoc*., **38**: 324-331.

Duke J (2011). Phytochemical and Ethnobotanical Databases. Available at: fromhttp://sun.arsgrin.gov: 8080/npgspub/xsql/duke/plantdisp.xsql?taxon=**209**. (Retrieved 05.02.11).

Edeoga HO, Okwu DE and Mbaebie BO (2005). Phytochemical constituents of some Nigerian medicinal plants. *African J. of Biotech,* **4**(7): 685-688.

Evans WC (1996). Pharmacognosy. 14th ed. W. B. Saunders Co. Ltd., Singapore, (9): 713-734

Garg V, Dhar VJ, Sharma A and Dutt R (2012). Facts about standardization of herbal medicines: A review. *J. Chin. Integr. Med*., **10**(10): 1022-1083.

Harborne JB (1984). Phytochemical Methods: A Guide to Modern Technique of Plant Analysis, 2nd ed. Chapman, p.17.

Harborne JB (1973). Phytochemical methods Chapman and Hall, Ltd., London, pp.49-188.

Kavit M, Patel B and Jain B (2013). Phytochemical analysis of leaf extract of *Phyllanthus fraternus*. *Res. J. Recent Sci*., **2**(1): 12-15.

Kokoski CJ, Kokoski RJ and Salma FJ (1958). Fluorescence of powdered vegetable drugs under ultraviolet radiation. *J. Am. Pharm. Assoc.,***47**: 715-717.

Kokate CK, Purohit AP and Gokhale SB Pharmacognosy (2008). Nirali Prakashan, Pune, India, 42 Edn., pp.A.1-A.2

Kulkarni YA, Yele SU, Gokhale SB and Surana SJ (2008). Pharmacognostical studies on leaves of *M. macrantha* Nees. *Pharmacog mag*, **4**(13): 83-88.

Lim TK (2012). *Carica papaya*. *In*: Edible Medicinal and Non-medicinal Plants, Vol.**3**: Fruits. Springer, New York, USA, pp.693-717.

Milind and Gurditta (2011). *Int. Res J. Pharm*., **2**(7): 6-12.

Mukherjee PK (2010). Quality Control of Herbal Drug, 1st Edition, Business Horizons Pharmaceutical Publishers, New Delhi, India, pp.184-191.

Nguyen TT, Parat MO, Shaw PN, Hewavitharana AK and Hodson MP (2016). Traditional aboriginal preparation alters the chemical profile of Carica papaya leaves and impacts on cytotoxicity towards human squamous cell carcinoma. *PLoS One*; **11**(2): e0147956.

Obdoni BO and Ochuko PO (2001). Phytochemical studies and comparative efficacy of the crude extracts of some Homostatic plants in Edo and Delta States of Nigeria. *Global J. Pure Appl. Sci*. **8**: 203-208.

Obianime AW and Uche FI (2008). The phytochemical screening and effects of methanolic extract of *Phyllanthus amarus* leaf on the biochemical parameters of male guinea pigs. *J. Appl. Sci. Environ. Manage*, **12**(4): 73-77

Olangunju JA, Adeneye AA and Fagbohunka BS (2009). Nephroprotective activities of the aqueous seed extract of *Carica papaya* Linn. in carbon tetrachloride induced renal injured Wistar rats: a dose- and time-dependent study. *Biol Med.*, **1**(1): 11-19.

Omojasola PF and Awe S (2004). The antibacterial activity of the leaf extract of *Anacardium occidentale* and *Gossyptum hirsutum* against some selected microorganisms. *Biosci. Res. Communication.,* **16**(1): 24-30.

Otsuki N, Dang NH, Kumagai E, Kondo A, Iwata S and Morimoto C (2010). Aqueous extract of *Carica papaya* leaves exhibits anti-tumor activity and immune-modulatory effects. *J. of Ethnopharmacology,* **127**(3): 760-767.

Owoyele BV, Adebukola OM, Funmilayo AA and Soladoye AO (2008). Anti-inflammatory activities of ethanolic extract of *Carica papaya* leaves. *Inflammopharmacol.*, **16:** 168-173.

Pokharkar RD, Saraswat RK and Kotkar S (2010). Survey of plants having antifertility activity from western ghat area of Maharashtra state. *J. Herb Med. Toxicol*., **4**(2): 71-75.

Raghuramalu N, Nair MK, and Kalyanasundaram SA (2003). Manual of laboratory techniques, second edition, National Institute of Nutrition, KMR, Hyderabad, p.201.

Reddy YSR, Vengatesh S, Ravichandran T, Subbaraju T and Suresh B (1999). Pharmacognostical studies of *Wrightia tinctoria* bark. *Pharmaceutical Biology*, **37**: 291-295.

Sama V, Swamy MM, Vijayalakshme S, Reddy YSR and Suresh B (1994). A report. Indian Drugs, **3**(4): 421-429.

Shahid-Ud-Duaula AFM, Anwarul and Basher M (2009). Phytochemical screening, plant growth inhibition, and antimicrobial activity studies of *Xylocarpus granatum. Malaysian. J. Pharm. Sci.* 7(1): 9–21.

Sofowara A (1993). Phytochemical screening of medicinal plants and traditional medicine in Africa, **2**nd Edition Spectrum Books Limited, Nigeria, pp.150-156.

Suresh K, Deepa K, Harisaranraj R and Vaira Achudhan V (2008). Antimicrobial and phytochemical investigation of the leaves of *Carica papaya* L., *Cynodon dactylon* (L.) Pers., *Euphorbia hirta* L., *Melia azedarach* L. and *Psidium guajava* L. *Ethnobot. Leaflets,* **12**: 1184-1191.

Trease GE and Evans MD (1989). A Textbook of Pharmacognosy, 13th edn. Builler Tindall and Caussel, London, pp.176-180.

Ugochukwu SC, Arukwe UI and Ifeanyi O (2013). Preliminary phytochemical screening of different solvent extracts of stem bark and roots of *Dennetia tripetala* G. Baker. *Asian J. of Plant. Sci. and Res.*, **3**(3): 10-13.

Van-Burden TP and Robinson WC (1981). Formation of complexes between protein and Tannin acid. *J. Agric. Food Chem*. **1**: 77.

Vasundhara S, Garima M, Akash S, Kamlesh K. and Vishwakarma (2013). A comparative study on quantitative estimation of tannins in Terminalia chebula, Terminalia belerica, Terminalia arjuna and Saraca indica using spectrophotometer. *Asian J. Pharm Clin Res*., **6**(3): p. 148-149.

Whistler RL and Bemiller JN (1993). Industrial Gums: Polysaccharides and their derivatives, Academic Press, San Diego, 318-337.

World Health Organization (2011). Quality control methods for herbal materials. Updated edition of Quality control methods for medicinal plant materials, 1998 1. Plants, Medicinal. 2. Medicine, Herbal. 3. Medicine, Traditional. 4. Quality control. 5. Manuals. I. World Health Organization. ISBN 978 92 4 150073 9 (NLM classification: QV 766).

Yasuma A and Ichikawa T (1953). A new histochemical staining method for protein. *J. Lab. Clin. Med*. Feb., **41**(2): 296-299.

Yogiraj V, Pradeep KG, Chetan SC, Anju G, and Bhupendra V (2014). *Carica papaya* Linn: An Overview. *Int. J. of Herb Med*., **2**(5): 01-08.

Zunjar V (2011). Pharmacognostic physiochemical and phytochemical studies *Carica papaya* Linn leaves. *Pharmacog J.,* **3**: 214-217.