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PHYTOCHEMICAL INVESTIGATION OF *CARICA PAPAYA* L. LEAVES EXTRACTS

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ABSTRACT

***Carica papaya* L. is powerhouse of nutrients. Different parts of papaya contain various types of metabolites. Owing to this *Carica papaya* L. leaves were screened for qualitative and quantitative analyses of phytochemicals. Polarity of solvents play pivotal role in phytochemical extraction. So plant leaves were extracted using five different solvents based on polarity. The solvents used were n-hexane, acetone, ethanol, methanol and distilled water. Phytochemical analyses of extracts revealed that water extract contained highest amount of alkaloids (5.72±0.08), and total phenolics (5.12±0.14). Highest content of tannins (0.13±0.003) and flavonoids (6.00±0.03) were observed in ethanolic and methanolic extracts respectively. Micro-nutrients (Fe, Mn, Zn, Cu) and macro-nutrients (P,K, Ca, Mg, Na) were detected in variable amounts in all extracts. Phytochemical composition of *Carica papaya* L. leaves extracts and effect of solvent on extraction of phytochemicals revealed by the current research work will be helpful for further isolation, purification related work.**

Key words: *Carica papaya* L., minerals, secondary metabolites, phytochemicals, leaves extracts, solvent polarity

I. INTRODUCTION

The vast and versatile pharmacological effects of medicinal plants are basically dependent on their phytochemical constituents mainly secondary plant metabolites. Secondary plant metabolites played an important role in alleviating several aliments in the traditional medicine and folk uses. In modern medicine, they provided lead compounds for the production of medications for treating various diseases ranging from migraine up to cancer (Hussein R A and El-Anssary A A 2018).

India is a country having a wide medicinal history as “Ayurveda”. Many plants and their parts have been explored and exploited for their medicinal importance. *Carica papaya* L. is not an exception. Various parts of it contain variety of primary and secondary metabolites. *Carica papaya* L. belongs to the plant family Caricaceae. The plant is weak, usually un-branched and having soft stem and can grow up to 20m high. *Carica papaya* L. plant bears cluster of large and long stalked leaves. Papaya is known as "A powerhouse of nutrients" and was reputably called the "The fruit of Angels" by Christopher Columbus in the 20th century (Saraf M and Kavimandan B 2018). During last few decades, accountable progress has been achieved regarding the biological activity and medicinal applications of *Carica papaya* L. and now it is considered as valuable nutraceutical fruit plant (Krishna K L *et al* 2008). Its leaf extract is now-a-days gaining popularity in many aspects. Various biological activities like anti-microbial, anti-oxidant, anti-pyretic etc. of *Carica papaya* L. leaves (Kirtikar K R and Basu B D 1998 and Krishna K L *et al* 2008) are mainly due to various biologically active components. Many biologically important compounds like primary metabolites, secondary metabolites (alkaloids, flavonoids, saponins, tannins etc), amino acids, dry matter, crude protein, crude fat, crude fibre, total ash, acid insoluble ash(Kirtikar K R and Basu B D 1998, Krishna K L *et al* 2008, Ayoola P B and Adeyeye A 2010, Saad R 2014, Marshall E U *et al* 2015, Akhila S and Vijayalakshmi N G 2015, Nath R and Dutta M 2016, Zunjar V 2016), nutritionally important minerals like phosphorous, sodium, potassium, calcium, iron, magnesium, zinc, copper, cadmium and nickel (Ayoola P B and Adeyeye A 2010, Sharma D K and Tiwari P B 2014, Verma K and Shrivastava V K 2014, Vyas S J *et*

al 2014, Fadare S *et al* 2015) and vitamins like C, A, B complex, and E; (Ayoola P B and Adeyeye A 2010, Begum M 2014 and Marshall U E *et al* 2015)are present in leaves of *Carica papaya* L.

Many researchers have done qualitative and quantitative estimation of primary and secondary metabolites and mineral estimation from extracts of *Carica papaya* L. leaves. Extraction of *Carica papaya* L. leaves is carried out using one or two solvents mainly ethanol/methanol/water (Farooq T 2009, Ayoola P B and Adeyeye A 2010, Bamisaye F A *et al* 2013, Sherwani K S *et al* 2013, Ndukwe O K *et al* 2013, Irondi A E *et al* 2013, Arumugam N *et al* 2014, Marshall E U *et al* 2015).

Similar polarity index containing solvents can dissolve phytochemicals that have similar or close related polarity index (Raman G *et al* 2005). *Carica papaya* L. leaves are rich source of wide range of phytochemicals. So using one or two solvents, it will not be possible to extract all kind of phytochemicals.

Hence, in the current research work we have done qualitative and quantitative estimation of secondary metabolites and mineral estimation from extracts of *Carica papaya* L. leaves using five solvents based on their polarity (non-polar to polar)- n-hexane, acetone, ethanol, methanol and water. The current research work will give useful information regarding suitable solvent for extraction of particular phytochemical component.

II. MATERIAL AND METHODS

All the chemicals and reagents used were of analytical Grade.

2.1 Collection of Plant:

Carica papaya L (Variety Taiwan 786) plantlets were obtained from Ram Biotech Nashirabad, Jalgaon and authenticated by expert botanist of the college. Thirty plantlets were grown in the college yard. These saplings were given organic manure (Biopower and Marvel, Nirmal, Chalisgaon) with fifteen days interval. Plants were watered as per requirement. Fresh, disease free, fifteen days old leaves of *Carica papaya* were collected for the research work.

2.2 Extraction of *Carica papaya* L.leaves:

Young and healthy leaves were washed thoroughly 2-3 times with running water followed by distilled water and finally shed air-dried. The leaves were finely powdered and used for extraction. The powdered samples (25gm/250ml) were extracted using Soxhlet apparatus in various solvents (n-hexane, acetone, ethanol and methanol) for 8 hours. Distilled water extract was obtained by macerating the powder on shaker (25gm/250ml) in distilled water. The extracts were concentrated using rotary vacuum evaporator (Evator).These extracts were used for further analyses.

2.3 Phytochemical screening:

The various phytochemical tests were performed in triplicates for establishing a profile of the extracts for their secondary metabolite composition. Alkaloids were detected by Dragendorff's test by adding 1ml sample to 1 to 2ml of Dragendorff's reagent. A prominent yellow precipitate indicated positive test. For saponin detection, extract (50 mg) was diluted with distilled water and made up to 20 ml. The suspension was shaken in a graduated cylinder for 15 min. A layer of foam was observed for presence of saponins. Phenolic compounds were detected by lead acetate test by adding 3ml sample to 3ml 10% lead acetate solution. A bulky white precipitate indicated the presence of phenolic compounds. Alkaline reagent test was used to detect flavonoids. Five ml filtrate was heated with 1ml NH_4OH solution. Yellow fluorescence indicated the presence of flavonoids (Begum M, 2014 and Saad R, 2014).Tannins were detected by adding 1ml sample, 1ml Folin-Denis reagent and 2ml 35% sodium carbonate solution. Blue colored indicated presence of tannin (Sadasivam S and Manickam A, 2005).

2.4 Estimation of secondary metabolites:

Estimation of all secondary metabolites mentioned below was done in triplicates.

Alkaloids were estimated (Irondi A E *et al* 2013) by adding 1ml sample, 1ml 0.025M $FeCl_3$ in 0.5M HCl and 1ml 0.05M 1,10-phenanthroline in ethanol. The mixture was incubated for 30 minutes in hot water bath maintained at 70°C ± 2°C temperature. Absorbance was read at 510nm. Colchicine was used as standard. The values were expressed as colchicine equivalent (CE) in mg/g of the extract.

Tannins were estimated (Sadasivam S and Manickam A 2005) by adding 1ml sample, 75ml water, 5ml Folin-Denis reagent and 10ml 35% sodium carbonate solution. Volume was made to 100ml using distilled water. After shaking well it was kept for incubation at room temperature for 30min in dark. Absorbance was read at 700nm. Tannic acid (5mg%) was used as standard and values expressed as tannic acid equivalent (TAE) in mg/g of the extract.

Total phenolic content was estimated (Irondi A E *et al* 2013) by adding 1ml sample, 5ml of Folin-Ciocalteu reagent, 4ml of 7.5% sodium carbonate solution and 1ml methanol. The test tubes were incubated for 30min at room temperature to complete the reaction. Absorbance was read at 765nm. Gallic acid

(1mg%) was used as standard and values were expressed as gallic acid equivalent (GAE) in mg/g of the extract.

Flavonoids were estimated (Irondi A E *et al* 2013) by adding 1 ml sample, 0.1ml 10% aluminum chloride, 0.1ml 1M potassium acetate and 2.8ml distilled water. Test tubes were incubated at room temperature for 30min. Absorbance was read at 415nm. Rutin (1mg%) was used as standard and values were expressed rutin equivalents (RE) in mg/g of the extract.

2.5 Estimation of minerals:

Minerals mentioned below were estimated by the methods given by Gupta P K (1999) in triplicates.

2.5.1 Sample preparation:

Extracts and crude powder were dried at 70°C in hot air oven until dry. It was then grinded in mixer and then these powdered samples were subjected for di-acid digestion. To 10ml acid mixture (10ml 60% perchlorate ($HClO_4$) + 50ml Conc. H_2SO_4), 0.2g sample was added. Solutions were placed at temperature 150°C initially for half an hour and when frothing subsided the temperature was increased to maximum 250°C to digest the sample. 20-50ml distilled water was added and filtered the solution through Whatmann's filter paper No. 40 and made up the volume to 100ml with distilled water. This solution was filtered through Whatmann's filter paper No. 1. Aliquots of these solutions were used for the determination of P, K, Na, Ca, Mg, Fe, Mn, Zn and Cu.

2.5.2 Estimation of Fe, Mn, Zn, Cu, Ca and Mg:

Iron, Manganese, Zinc, Copper, Calcium and Magnesium were estimated by Atomic absorption spectroscopy (Chemito AA 201). To determine the concentration of minerals, di-acid digest was further diluted 25-50 times depending upon the concentration of the minerals. The diluted digests were fed to the atomic absorption spectrophotometer (AAS) using the specifications for the specific instrument used. The determination of Fe as also of Mn, Zn and Cu was done by using AAS with the following specifications for mono-element hollow cathode lamp. The exact specifications were as per the particular instrument used. For determination of Zn, instead of hollow cathode lamps, Electrode less Discharge Lamp (EDL) was used. It provided better sensitivity and detection at lower concentrations. For determination Ca, Mg, Fe, Mn, Zn and Cu, 100ppm solution of Calcium carbonate, Magnesium sulphate ($MgSO_4.7H_2O$), Ammonium-ferro-sulphate ($(NH_4)_2Fe(SO_4)_2.6H_2O$), Manganese sulphate ($MnSO_4.H_2O$), Zinc sulphate ($ZnSO_4.\ 7H_2O$) and Copper sulfate ($CuSO_4.5H_2O$) was used as standard respectively.

2.5.3 Estimation of Na and K:

Sodium and potassium were determined by flame photometry (Systronics, Flame photometer 130). Di-acid digested sample was diluted with deionized water to the suitable concentration range.The samples were then read in flame photometer using filter for Na and K. NaCl and KCl (100ppm) were used as standards.

2.5.4 Estimation of P:

Phosphorus was determined by UV spectrophotometry (UV 1800 Shimadzu). To 1ml di-acid digested sample 1.0ml 10% TCA (Trichloroacetic acid), 0.4ml molybdate solution, 0.2ml ANSA (Amino Naphtho Sulfonic Acid) reagent and 4ml double distilled water were added and kept at room temperature for 5min. Blue color developed was measured at 640nm. Potassium dihydrogen phosphate (0.007 M) was used as standard.

III. RESULTS AND DISCUSSION

3.1 Yield of extracts:

The crude extract quantity, purity, and quality greatly depend on the plant part used and the solvent used for the extraction (Wakeel A *et al* 2019). Yield and changes in colour of the extracts after completion of reaction with the suitable reagents were recorded. All the extracts were found to be sticky. The color of the extract varied from green to brownish green with respect to the solvent used.

Table-1: Yield of *Carica papaya* L. leaves extracts

Sr. No.	Solvent	Yield of dry extract (gm/100gm)
1	n-hexane	04.00
2	Acetone	12.50
3	Ethanol	14.00
4	Methanol	17.39
5	Distilled water	16.25

Moreover, the polarities of the solvents, the chemical compositions of the sample also determine the yield of extraction (Tay Z H and Chong K P 2016). The results also showed that the extraction yields obtained was affected by the solvent used. High yield of extract was obtained in polar solvents as compared to non-polar solvents. The polar solvents are able to increase cell permeability and to penetrate inside the cells, therefore, extracting more endocellular secondary metabolites, both polar and less polar compounds compared to the use of non polar solvents, such as n-hexane (Yusnawan E 2013). The differences in extraction yields of various solvents might be due to differences of protic solvent character on diffusivity and solubility of solutes in solvent during extraction (Nguyen V T and Scarlett C J 2016). Amongst various solvents used for extraction, maximum yield was obtained in methanol which might be due to solubility of endogenous compounds along with phenols. It is likely that papaya leaves contain more polar than non-polar compounds (Tay Z H and Chong K P 2016). These results indicated that the extraction yield in *Carica papaya* L. leaves was greatly affected by solvents, of which methanol was the most effective solvent for extraction of solutes from *Carica papaya* L. leaves.

Similar results extraction yield in *Carica papaya* L. leaves were obtained by Asghar N *et al*, (2016) with yield minimal in hexane (3.95%) and maximal in water (28%) extracts. Varying degree of percent yields during extraction of *Carica papaya* L. leaves (ethanol 12.44%, methanol 3.66% and water 4.22%) was obtained by Pandey S *et al*, (2016). Yusha'u M *et al*, (2009) obtained maximum yield of *Carica papaya* L. leaves extract in ethanol (6.25%) whereas Saini R *et al*, (2016) obtained least yield in chloroform extract (3.6%) and maximum yield of *Carica papaya* L. fruit extract in hydro-alcohol (43.20%gm).

3.2 Phytochemical analyses:

The aim of phytochemical analyses was to determine secondary metabolites from the polar and non polar extracts of the *Carica papaya* L. leaves.

Table-2: Qualitative phyto-chemical analyses of *Carica papaya* L. leaf extracts

Tests	n-Hexane	Methanol	Water	Ethanol	Acetone
1] Alkaloids	+	+	+	+	+
2] Saponins	–	+	+	+	+
3] Phenolic Compounds	+	+	+	+	+
4] Tannins	+	+	+	+	+
5] Flavonoids	+	+	+	+	+

+ indicates presence and – indicates absence

The result stated the presence of alkaloids, phenolic compound, tannins and flavonoids in all the extracts. Whereas saponins were absent in n-hexane extract while were present in all other extracts.
Yusha'u M *et al*, (2009), Saad R *et al*, (2014), Marshall E U *et al*, (2015), Nariya A and Jhala D S (2017) revealed that the *Carica papaya* L. leaf extracts contained bioactive compounds like alkaloids, flavonoids, phenols, steroids, glycosides, carbohydrates, tannins and saponins.

The presence of phytosterol was very prominent in all extracts of *Carica papaya* L. leaf, along with saponins, glycosides, proteins and amino acids, flavonoids, terpenoids (Farooq T *et al*, 2009, Sherwani K S *et al*, 2013 and Raaman N 2015). In *Carica papaya* L. leaf aqueous extract other important constituents namely volatile oil, proteins, amino acids and starch were found to be absent by Saini R *et al*, (2016). The phytochemical screening and percentage estimation (quantitative) of various solvent crude extracts of shoot and leaf of *Carica papaya* L. showed the presence of flavonoids, saponins, glycosides, steroids, terpenoids, napthoquinones, anthrocyanin, leucocyanin, coumarins, phenols and tannins (Arumugum N *et al*, 2014).

Biochemical content of leaves is affected by environment, climate, earth texture, and weather (Widhyavati P S *et al* 2014). Moreover, polar solvents extract polar compounds while less polar solvents extract non-polar compounds. Polar compounds are also easier to extract compared to non-polar compound (Tay Z H and Chong K P 2016). A good solvent is characterized by optimal extraction and its capacity in conserving the stability of chemical structure of desired compound (Thouri A *et al* 2017). So quantitative estimation was done for alkaloids, tannins, total phenols, and flavonoids. The type of solvent and its polarity have a significant impact on levels of poly phenols, flavonoids, tannins and alkaloids. It was found that water extract had highest alkaloid content amongst the five extracts whereas tannins, total phenolic content and flavonoids were found to be maximum in methanol extract. It was elucidated that the water, ethanolic and methanolic (all proton donars) extracts showed higher phenolic content than acetone and n-hexane (proton acceptor) extracts, because of their lower efficiency of solubility (Thouri A *et al* 2017). MINITAB version 18 software was used for statistical analysis of secondary metabolites. Data was analysed using ANNOVA: One-Way analysis of variance using Tukey test. Level of significance was set at 0.05. P value of all secondary metabolites except tannins analysed was found to be less than 0.05 ($P<0.05$) while P value for tannin was found to be 0.445 ($P>0.05$). Statistical analysis also indicated that solvent has significant effect on extraction of secondary metabolites except for tannins.

Table-3: Quantitative Phytochemical analyses of *Carica papaya* L. leaf extracts

Phytochemicals	n-hexane	Acetone	Ethanol	Methanol	Water
Total alkaloids (mg CE/g)	3.23 ±0.19	3.61 ±0.29	4.23 ±0.69	4.83 ±0.10	5.72 ±0.08
Tannins (mg TAE/g)	0.06 ±0.005	0.10 ±0.003	0.17 ±0.001	0.16 ±0.002	0.13 ±0.003
Total phenolic compounds (mg GAE/g)	3.76 ± 0.05	4.13 ±0.06	4.72 ±0.05	4.89 ± 0.01	5.12 ±0.14
Total Flavonoids (mg RE/g)	4.80 ±0.04	5.09 ±0.04	5.38 ±0.05	6.00 ±0.03	4.29 ±0.05

The salts of alkaloids are soluble in water or dilute acids, whereas they are insoluble or sparingly soluble in organic solvents. The differences in the solubility of alkaloids, depends on their form (Kukula-Koch W A and Widelski J 2017). Aquadest could dissolve alkaloid and glycoside compounds, but ethanol was effective to extract flavonoid, phenolic compounds and alkaloids from *Pluchea indicia Less* leaves (Widhyavati P S *et al* 2014). The polarities of the poly-phenols range from polar to non-polar, optimum extraction of poly-phenols (flavonoids, tannins and all other) is usually obtained in the polar solvent which have a better efficiency of solvation as a result of interactions (hydrogen bonds) between the polar sites of the antioxidant compounds and the solvent than non-polar one (Thouri A *et al* 2017). Phytochemical compounds in methanolic extract were found to be potential in donating hydrogen atom so that these compounds could form complex compounds with aluminium ion at total flavonoid assay (Tapas A *et al* 2008 and Amic D *et al* 2003).

Varied concentrations of secondary metabolites have been reported by many researchers. The presence of alkaloids, tannins, total phenols and flavonoids found in *Carica papaya* L. leaves and seeds were in the following range; 0.05mg/g to 16.91mg/g; 0.001mg/g to 3.1mg/g; 0.011mg/g to 2.6mg/g; 0.013mg/g to 7.23mg/g respectively (Afolabi I S *et al*, 2011, Irondi A E *et al*, 2013; Ndukwe O K, 2013, Marshall E U *et al*, 2015, Bamisaye F A, 2013, Faeji C O *et al*, 2017, Nugroho A *et al*, 2017).

Table-4: Mineral estimation of *Carica papaya* L. leaf extracts and powder (mg/100gm)

Extracts	Macronutrients					Micronutrients			
	P	K	Ca	Mg	Na	Fe	Mn	Zn	Cu
Acetone	17.47 ±0.45	3398.79 ±3.20	132.69 ±0.95	320.85 ±0.77	706.02 ±0.50	51.83 ±0.12	3.61 ±0.13	8.29 ±0.11	29.97 ±0.07
Ethanol	2.67 ±0.21	62.03 ±0.56	65.41 ±0.49	229.81 ±0.50	398.50 ±0.59	37.45 ±0.03	1.22 ±0.03	5.01 ±0.11	16.24 ±0.03
Methanol	5.82 ±0.44	1399.75 ±0.88	63.40 ±0.45	316.44 ±0.61	411.98 ±0.63	61.53 ±0.32	2.41 ±0.05	9.87 ±0.04	15.63 ±0.05
Water	8.72 ±0.56	1814.55 ±0.60	231.65 ±0.58	208.94 ±0.69	373.23 ±0.61	27.58 ±0.24	2.14 ±0.09	9.30 ±0.06	44.12 ±0.25
n-hexane	3.81 ±0.56	110.16 ±0.81	187.34 ±0.71	272.18 ±0.64	330.22 ±0.73	49.63 ±0.21	2.81 ±0.11	5.73 ±0.09	61.23 ±0.32
Powder	10.27 ±0.46	1924.00 ±2.08	120.84 ±0.61	288.80 ±0.55	307.00 ±1.00	50.60 ±0.23	5.85 ±0.10	6.98 ±0.16	13.29 ±0.28

For the first time, this research article is reporting estimation of minerals from *Carica papaya* L. leaves extracts. As per authors' information, there are reports on mineral estimation from *Carica papaya* L. leaves and powder but not from extracts.

Plants obtain minerals from the soil and water but its concentration varies with environmental, climatic or geographic conditions. Minerals from the plant sources may also vary from place to place, because soil mineral content varies geographically (Sharma D K *et al* 2013). The obtained values of various nutrients are from the extracts and powder of *Carica papaya* L. leaves. Significant differences in mineral content were observed between all the samples. Concentration of phosphorus, potassium, magnesium and sodium were found to be highest in acetone extract. Highest values of calcium, iron, manganese, zinc and copper were found in water, methanol powder, methanol and n-hexane extracts respectively. Minimum quantity of phosphorus, potassium, calcium, magnesium, sodium, iron, manganese, zinc and copper were observed in ethanol, ethanol, methanol, water, powder, water, ethanol, ethanol and powder respectively. Amongst macronutrients potassium was predominant while phosphorus was found to be lowest. Among micronutrients iron was predominant while manganese was found to be lowest.

MINITAB version 18 software was used for statistical analysis of macro and micro-nutrients. Data was analysed using ANNOVA: One-Way analysis of variance using Tukey test. Level of significance was set at 0.05. P value of all macro and micro-nutrients analysed was found to be less than 0.05 ($P<0.05$). Statistical analysis also indicated that solvent has significant effect on extraction of macro and micro-nutrients.

As per Maisarah A M *et al* (2014) research work, potassium, calcium and magnesium were the predominant elements while iron was analyzed to be the lowest content in papaya leaves. However in the study of Ayoola P B and Adeyeye A (2010) mineral analysis showed high values of Ca, Mg, Na, K, Mn, in the green leaves of *Carica papaya* L., but Fe in yellow leaves of *Carica papaya* L. were more as compared with other. Thus green pawpaw leaves gave a source of essential nutrients while yellow pawpaw leaves were a source of iron.

Various proportions of K, Na, Mn, Mg, Fe, Zn, Cu, Ca, Cd and Ni were reported by Fadare O A *et al* (2015) in *Carica papaya* L. leaves. *Carica papaya* L. (green, yellow and brown) leaves collected from plain as well as hill area of Northern India were found to be rich source of various minerals like Ca, Mg, Mn, Na, K, Fe, Zn, Cu, Cr, etc (Sharma D K *et al* 2013).

IV. CONCLUSION

Carica papaya L. leaves extracts are rich source of various secondary metabolites, macro and micro-nutrients having wide range of applications in various fields like agriculture, medicine etc. Information on effect of solvent on extraction of biological components will be useful for isolation and purification of it. .

Author's contribution:

Conceptualization and designing of the research work (Bhushan R. Kavimandan); Execution of field/lab experiments and data collection (Mrinal S. Saraf); Analysis of data and interpretation (Bhushan R. Kavimandan and Mrinal S. Saraf); Preparation of manuscript (Bhushan R. Kavimandan and Mrinal S. Saraf).

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