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Development of quality standards of *Carica Papaya* Linn. leaves

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ABSTRACT

The present study was performed for the development of physico-chemical parameters of Carica papaya Linn. leaves belonging to family Caricaceae. The study comprises physico-chemical and phytochemical evaluation to confirm purity and authenticity of Carica papaya leaf based on WHO guidelines. Microscopy of the leaf showed presence of epidermis, collenchyma, and parenchyma, scellerenchyma, xylem, phloem and pith was found to be absent. Successive extractive value is found highest in petroleum ether extract 20.44 % (on dry weight basis). Mean ash values (%) are 16.72 (total), 3.25 (acid insoluble ash), and 6.05 (water soluble ash) and moisture content is found to be 7.77 % and the phytochemical analysis indicate the presence of carbohydrates, terpenoids, flavonoids, phenolic compounds in different extracts of C.papaya L. leaf. TLC fingerprinting profile of different extract was also developed which exhibited presence of several medium polar compounds.

Keywords: *Carica papaya*, Caricaceae, papaya, phytochemical screening, WHO guidelines.

INTRODUCTION

Carica papaya Linn belonging to family Caricaceae is commonly known as papaya in English, Papita in Hindi and Erandakarkati in Sanskrit [1, 2, 3]. The plant is native to tropical America [4] and was introduced to India in 16th century. The plant is recognised by its weak and usually unbranched soft stem yielding copious white latex and crowded by a terminal cluster of large and long stalked leaves, is rapidly growing and can grow upto 20 m tall [5, 6]. Traditionally leaves have been used for treatment of a wide range of ailments, like in treatment of malaria [7], dengue, jaundice [8], immunomodulatory and antiviral activity [9].

Young leaves are rich in flavonoids (kaempferol and myricetin) [10], alkaloids (carpaine, pseudocarpaine, dehydrocarpaine I and II) [11], phenolic compounds (ferulic acid, caffeic acid, chlorogenic acid), the cynogenetic compounds (benzylglucosinolate) found in leaves [12]. Both leaf and fruit of the *C.papaya* L. possess carotenoids namely β- carotene, lycopene [13], anthraquinones glycoside [13], as compared to matured leaves and hence possess medicinal properties like anti-inflammatory [14] hypoglycaemic [15], antifertility [16], abortifacient [17], hepatoprotective [18], wound healing [19], recently its antihypertensive [20] and antitumor [21] activities have also been established. Leaves being an important part of several traditional formulations are undertaken for standardization for various parameters like moisture content, extractive values, ash values, swelling index, etc.

MATERIALS AND METHODS

Plant collection

Young leaves of *Carica papaya* Linn were collected in the month of July 2012 from Jamia Hamdard campus, New Delhi, authenticated by Dr. H. B. Singh, Scientist F and Head, Deptt. of Raw material and Herbal museum. NISCAIR, Pusa Campus, New Delhi. A voucher specimen (NISCAIR/RHMD/Consult/-2012-13/2158/164) was deposited in the same department, NISCAIR, New Delhi.

The leaves were washed with water, shade dried followed by drying in oven at 35°C for two days. The drug was then powdered removing the stalk and woody part, then kept in air tight container at room temperature away from moisture for further study.

Morphological studies
Papaya fruits were examined to study morphological and organoleptic characters. Sample for microscopy were prepared by embedding in formalin, glycerine, water (8:1:1) for a week. The sections were cut by razor. The cut sections were seen under microscope (Motic of B1 series) at 10x, 40x, 100x after staining with Phloroglucinol and HCl [22].

Physicochemical standardization
Extractive values were determined for cold, hot and successive extraction methods where 4 gm of coarse powder sieved with 40 mesh size was dissolved in 100 ml of solvent (from non polar to polar). Standard methods were followed to determine the total ash, acid-insoluble ash and water soluble ash values, loss on drying, was determined according to WHO guidelines [22].

Determination of pH
pH 1% solution
Accurately weighed (1 gm) powder drug was dissolved in accurately measured 100 ml of distilled water, filtered and checked the pH of filtrate with a standard glass electrode.

pH 10% solution
Accurately weighed (10 gm) powder drug was dissolved in accurately measured 100 ml of distilled water, filtered and the pH of filtrate was checked with a standard glass electrode.

Loss on drying
An accurately weighed (2 gm) shade dried leaf powder of *C. papaya* L. was taken in tarred evaporating disc. The crude drug was heated at 105°C in an oven till a constant weight was obtained. Percentage moisture content was calculated with reference to the shade dried material [23].

Determination of foaming index
About 1 g of plant material was reduced to a coarse powder, weighed accurately and transferred at moderate boiling for 30 minutes. Cooled and filtered into 100 ml volumetric flask. The detection was poured into 10 ml and adjusted the volume of liquid in each tube with water to 10 ml. Stoppered the tubes and was shaken them in a lengthwise motion for 15 sec; two shakes per second. Allowed to stand for 15 minutes and the height of foam were measured [22].

Determination of swelling index
Specified quantity of the plant material (3 gm) concerned previously reduced to the required fineness and accurately weighed taken into 25 ml glass stopper measuring cylinder. 25 ml of water added and the mixture was shaken thoroughly every 10 minutes for 1 hour. It was allowed to stand for 3 hours at room temperature. The mean value of the individual determinations was calculated related to 1 gm of the plant material [22].

Determination of Resin content
The accurately weighed leaf drug (5 gm) was rapidly refluxed with acetone (3× 200 ml) for 6 hours and the drug was exhausted for resin content. The excess solvent was removed by distillation on a water bath. The residue so obtained was suspended in water and transferred to separating funnel repeatedly extracted with solvent ether (2× 200 ml) to extract all resin. The ether extract was cooled over anhydrous sodium sulphate and excess ether removed over water bath. Residue was transferred to a weighed beaker and final weight was noted with reference to air dried drug material.

Fluorescence Analysis
The fruit powder was subjected to fluorescence analysis after being separately treated with water, NaOH, H_2SO_4, HCl, HNO_3, chloroform, ferric chloride, ammonia solution and picric acid. Since many herbs fluorescence when powder is exposed to UV light and this can help in their identification method. The fluorescence character of the plant powder was studied both in day light and UV light (254 and 366 nm) [24].

Powder drug reaction with different reagents

The powdered drug was treated separately with different reagents and acids like water, NaOH, H_2SO_4, HCl, HNO_3, chloroform, ferric chloride, ammonia solution and picric acid, the colour shown by that treatment was noted as such and under the microscope [25].

Phytochemical screening

The phytochemical evaluation of drug was carried out as per the method described. Previously dried powdered fruits (5 gm) were extracted in a Soxhlet apparatus with petroleum ether, ethyl acetate, chloroform, acetone, methanol, hydroalcoholic and water successively. The extracts were evaporated to dryness under vacuum. These extract were used for the analysis of different phyto-constituents viz. alkaloids, carbohydrates, phenolics, flavonoids, proteins, amino acids, saponins, mucilage and resins and lipids etc [23].

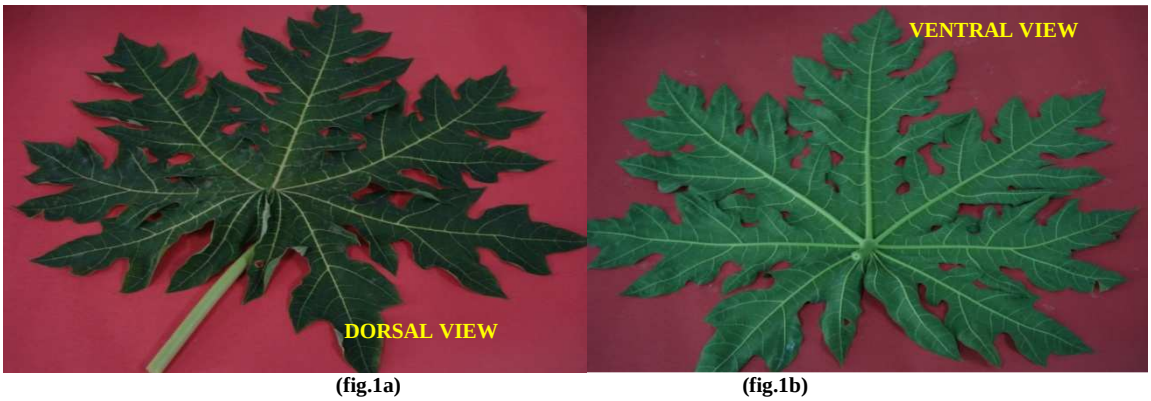
TLC/HPTLC fingerprinting

TLC finger printing profile was done for petroleum ether, ethyl acetate, chloroform, acetone and alcoholic extracts to find out the nature of compounds present. The solvent system used was *n* Hexane: Acetone (8.5:1.5), 10mg/ml of leaf sample of different extracts was procured from hot extraction method and stock solution of 100 µg/ml of β-carotene (Sigma Aldrich, New Delhi) using chloroform as a solvent was prepared. Test solution and standard solution was applied on a precoated silica gel 60 F_{254} TLC plate and run in the previously saturated solvent system. After development the plates are visualized after spraying with Anisaldehyde in Conc H_2SO_4 followed by heating at 105°C for 5 min [26].

RESULTS AND DISCUSSION

Macroscopic characters

Physical examination of the untreated sample of *Carica papaya* L. leaves was carried out under diffused sunlight and artificial source similar to day light. Morphological examination revealed that the leaves of papaya are usually larger and arranged spirally having long stem (1-3.5 ft) and their blades are split into 7-11 main lobes are usually veins are deeply palmated and serration are shallow. The stem have a green to dark green colour while leaves are green with yellow ribs and veins. The petiole develops last in leaf is solid and cylindrical but hollow. According to pinnate or palmate type of veination the incision found to be palmatipartite shown in Fig 1a and Fig 1b. Organoleptic property of leaf powder is green to dark green in colour, with smooth surface. The drug powder is irritating with characteristic odour and bitter in taste.



(fig.1a) **(fig.1b)**

Fig 1: Macroscopic characters of *Carica papaya* L. leaf

Microscopy of leaf

Transverse section of the leaf shows a well defined upper and lower epidermis surrounded by well defined 5-7 layers of collenchyma and scellerenchyma. The epidermis is composed of very large round cells with wavy and refractive walls. The inner walls of the cells are strongly but unevenly thickened. The endodermis is composed of parenchymatous cells with moderately thickened walls and are found usually attached to the portions of the parenchyma the cells of which are small, thin walled and polygonal. The pith is found to be absent as the stalk is hollow from inside. A middle portion is covered with xylem and phloem surrounded by parenchymatous cell that in turn surrounded by scellerenchyma cells (Fig. 2a, 2b, 2c, 2d, 2e). The observed characteristics of the leaves were found to be in complete agreement with previous finding.

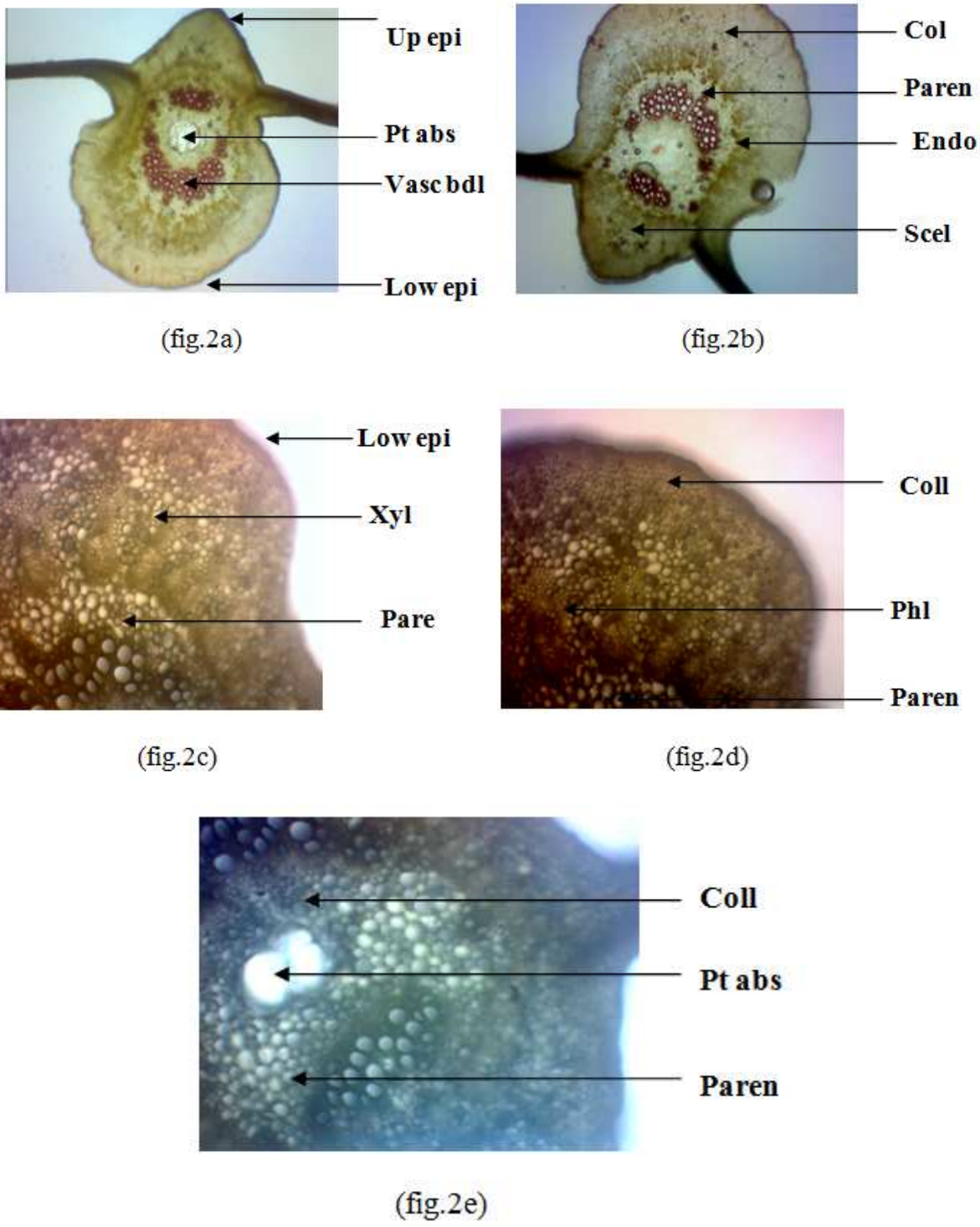


Fig 2: Transverse section of Papaya leaves

(abbreviations: up epi: upper epidermis; pt abs: pith absent; vasc bdl: vascular bundle; low epi: lower epidermis; paren: parenchyma; scel: sclerenchyma; endo: endodermis; xyl: xylem; phl: phloem; coll: collenchyma).

Extractive value

The extractive values were studied on dried leaf powder as per the procedure described above. All the values were taken in triplicate (Table 1 & Fig 3).

Table 1: Extractive values of *Carica papaya* Linn. Leaf

Extractive values	PE	EA	CHL_3	ACT	Alc	Hyd-Alc	Aqs
Cold maceration	16.88	14.97	12.67	12.26	15.66	13.43	10.58
Hot extractive	25.36	10.08	25.07	17.28	20.46	50.15	25.51
Successive extractive	20.44	05.00	10.92	15.85	10.75	20.04	19.18

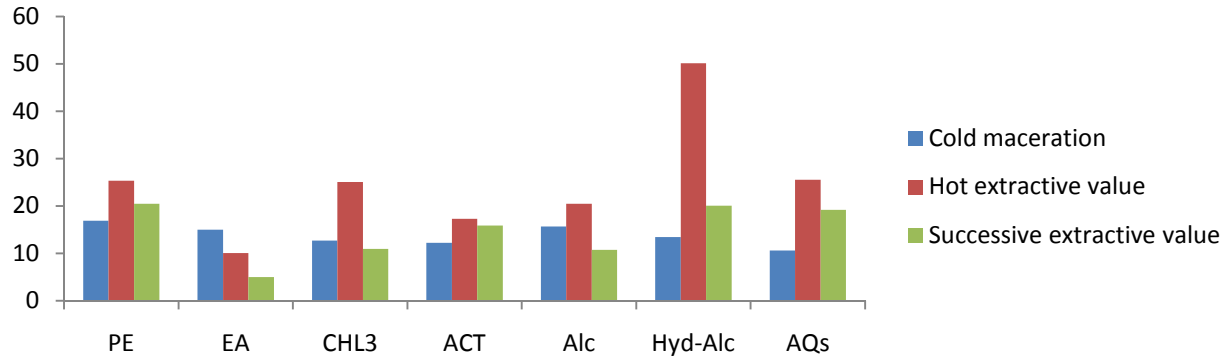


Fig 3: graphical representation of different extractive values of *Carica papaya* L. leaves

Ash values

The total ash value, acid insoluble ash value and water soluble ash value were found to be 17.23 %, 3.25 % and 6.05 % w/w respectively. Ash value is useful in determining authenticity and purity of drug and these values are important quantitative standards.

Fluorescence analysis

The powder of the fruit of *C. papaya* L. (mesh size 40) was examined under day light and UV light (Table 2).

Table 2: Fluorescence analysis of leaf powder

S.no	Chemical treatment	day light	UV 254nm	UV 366nm
1	Distilled water	Green	Dark green	Brown
2	NaOH (1N)	Light green	Dark green	Green
3	Conc H_2SO_4	Brownish green	Dark brown	Green
4	Conc HCl	Green	Black	Dark green
5	Conc HNO_3	Brown	Black	Dark green
6	Ferric chloride (5%)	Yellowish green	Black	Light brown
7	Petroleum ether	Dark green	Green	Dark green
8	Picric acid	Light green	Green	Dark green
9	KOH (1%)	Light green	Black	Light brown

Table 3: Treatment of leaf powder with different reagent

S.no	Treatment with reagent	Colour analysis	S.no	Treatment with reagent	Colour analysis
1	Iodine	Brown	**6**	Conc HNO_3	Reddish brown
2	Ethanol	Green	**7**	Conc H_2SO_4	Brown
3	Ferric chloride (5%)	Light brown	**8**	Conc HCl	Green
4	KOH (1%)	Green	**9**	Petroleum ether	Green
5	NaOH (1N)	Green	**10**	Picric acid	Green

Table 4: Phytochemical screening of leaf using different extracts

S.NO	TESTS	PE	EA	$CHCl_3$	ACE	MeOH	HA	AQS
1	**Alkaloids**	_	+	+	++	_	++	+++
2	**Carbohydrates**	_	+	_	_	_	+	+
3	**Saponins**	_	+	_	+	+	+	++
4	**Glycosides**	+	+	_	+	+	+	_
5	**Proteins**	_	+	_	+	+	_	_
6	**Steroids**	+	+	+	+	+	+	_
7	**Phenolics**	+	+	++	++	+++	++	+
8	**Flavonoids**	_	+	_	+++	++	+	_
9	**Terpenoids**	++	+	_	++	+	+	_
10	**Tannins**	+	_	_	_	+	+	+

(Note: +++: strongly present; ++: present; +: poorly present; - : absent)

Foaming index
The height of the foam in every test tube was found to be less than 1 cm, so the foaming index was less than 100.

Swelling index
The swelling index was found to be less than 100.

Loss on drying
The mean loss on drying was found to be 7.77 %.

Resin content
The mean resinous matter was found to be 10.54 %.

pH values
The mean pH value of 1 % solution and 10 % solution was found to be 6.78 and 5.60, respectively.

TLC/ HPTLC fingerprinting
The weighed quantity of fruit was extracted in a Soxhlet apparatus for 6 h using twice the amount of solvent (pet ether, ethyl acetate, chloroform, acetone and methanol) at a controlled temperature. The extract was dissolved in the respective solvent (4 mg/ml). The spots were applied with the help of Linomat syringe using Linomat applicator and developed in optimized solvent system (*n*-hexane: acetone :: 8.5:1.5). Developed plate was derivatized with anisaldehyde-sulphuric acid reagent, dried at 105 ºC for 5 minutes and observed in day light and UV (Fig 4 & Table 5a, 5b).

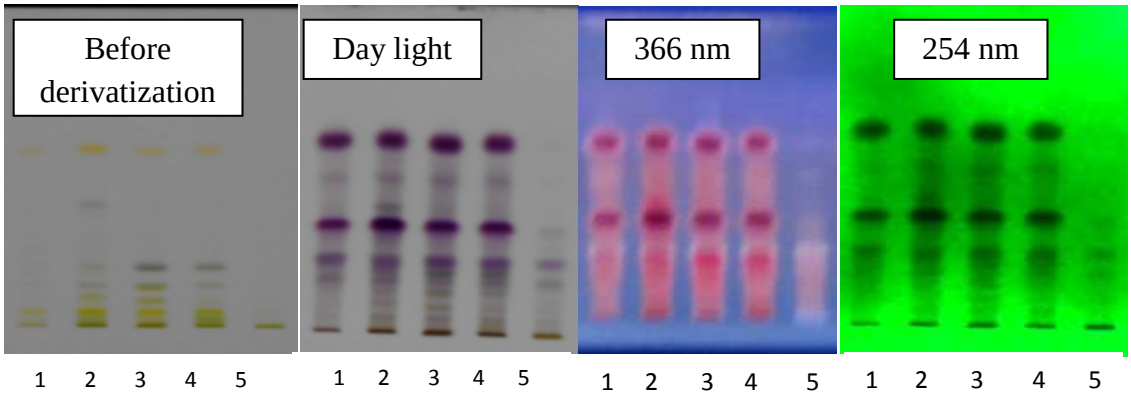


Fig 4: HPTLC plates before and after derivatization in day light, 366 nm, 245 nm.

Table 5 (a): TLC fingerprinting profile of *Carica papaya* leaf extracts before derivatization

S.no	Extract	No of spots	rf values
1	**PL (pet ether leaf)**	5	0.16, 0.24, 0.30, 0.36, 0.71
2	**EL (Ethyl acetate leaf)**	8	0.12, 0.16, 0.24, 0.30, 0.36, 0.45, 0.64, 0.71
3	**CL (chloroform leaf)**	6	0.10, 0.16, 0.24, 0.30, 0.36, 0.71
4	**AL (acetone leaf)**	4	0.16, 0.23, 0.31, 0.36, 0.44
5	**ML (methanol leaf)**	0	-

Table 5 (b): TLC fingerprinting profile after derivatization in day light, 254 nm and 366 nm

S.no	Extract	(No of spots) R_f values in day light	(No of spots) R_f value at 254 nm	(No of spots) R_f value at 366 nm
1	**PL (pet ether leaf)**	(6) 0.16, 0.24, 0.30, 0.36, 0.71	(4) 0.24, 0.36, 0.44, 0.72	(6) 0.16, 0.24, 0.30, 0.36, 0.48, 0.71
2	**EL (Ethyl acetate leaf)**	(8) 0.12, 0.16, 0.24, 0.30, 0.36, 0.45, 0.64, 0.71	(8) 0.12, 0.24, 0.30, 0.36, 0.45, 0.49, 0.64, 0.72	(8) 0.16, 0.24, 0.30, 0.35, 0.49, 0.54, 0.64, 0.71
3	**CL (chloroform leaf)**	(6) 0.10, 0.16, 0.24, 0.30, 0.36, 0.71	(8) 0.12, 0.16, 0.23, 0.30, 0.36, 0.44, 0.64, 0.72	(10) 0.10, 0.16, 0.23, 0.29, 0.36, 0.45, 0.49, 0.62, 0.64, 0.71
4	**AL (acetone leaf)**	(5) 0.16, 0.23, 0.31, 0.36, 0.44	(7) 0.16, 0.23, 0.31, 0.44, 0.51, 0.63, 0.71	(8) 0.16, 0.23, 0.30, 0.36, 0.45, 0.49, 0.63, 0.71
5	**ML (methanol leaf)**	(4) 0.30, 0.36, 0.44, 0.71	(1) 0.35	(3) 0.10, 0.16, 0.24

DISCUSSION

Carica papaya Linn was evaluated for physico-chemical and phytochemical analysis by using different organic solvents to determine the type and amount of soluble constituents present in given amount of medicinal plant material. Phytochemical analysis was performed on different leaf extracts confirmed the presence of alkaloids, glycosides, saponins, tannins, flavonoids, phenolic, proteins, amino acids etc. TLC fingerprinting helped in conformation of presence of different constituent depending on the polarity of the constituents which are exhibited as number of resolved bands.

CONCLUSION

This research report will envisage the existing knowledge regarding *Carica papaya* L. leaves to develop quality control of various herbal formulation containing leaves as main ingredients. This information can be useful in distinguishing and determining type of compounds present in papaya leaf as well as set the standards for future researches.

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