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Pharmacognostic investigations on the seeds of *Carica papaya* L.

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Abstract

Carica papaya L. belongs to the family Caricaceae and is commonly known as papaya. All parts of the plant are traditionally used for curing various diseases and disorders. It is a tropical fruit well known for its flavor and nutritional properties. Unripe and ripe fruit of papaya is edible but the seeds are thrown away. Instead, they can be therapeutically used. Natural drugs are prone to adulteration and substitution; to prevent it, it is always essential to lay down quality control and standardization parameters. Hence, the objectives of the present work were pharmacognostic, physicochemical and phytochemical studies of *Carica papaya* L. un-ripe and ripe seeds. Macroscopic, microscopic and powder features, phytochemical, physicochemical properties and fluorescence characteristics were determined using standard methods. The seeds were of clavate shape, hilum was of wavy type, margin was smooth, unripe seed was creamiest white in colour while ripe seed was dark black in colour. The microscopic study showed seed was divided into four parts epicarp, mesocarp, testa and endocarp. The epicarp was single layered with thin smooth cuticle layer, polygonal parenchymatous cells. Endocarp consisted of sclerenchyma cells with oil globules, The plasmodesma was yellowish in colour with radially elongated thick walled mucilaginous cells. The endocarp consisted of pitted aleuronic grains, oil globules, crystals of calcium oxalate and dicotyle structure. The un-ripe seed powder showed oil granules, sclerenchyma cells, mesocarp, square calcium oxalate crystals, vittae, etc while ripe seed showed sclerenchyma cells, oil globules, mesocarp, endosperm with aleurone grains, vittae, square calcium oxalate crystal, etc. The parameters evaluated in physicochemical analysis were all within limits. All the extractive values of un-ripe seed were more than that of ripe seed. The crude powder of un-ripe seeds showed presence of alkaloids, phenols and saponins while ripe seed showed presence of alkaloids. The solvent extracts revealed maximum amount of phytoconstituents in aqueous extract than in organic solvent extracts. The parameters evaluated in this study are the diagnostic features of the seeds and can be useful in identifying the genuine drug from adulterated drug.

Keywords: *Carica papaya*, caricaceae, pharmacognostic, physicochemical, phytochemical, un-ripe seed

1. Introduction

Caricaceae family belongs to the order Brassicales; there are six genera in this family viz. *Carica*, *Cylicomorpha*, *Horovitzia*, *Jacaratia*, *Jarilla*, *Vasconcellea* and about 25 to 28 species. The genus *Carica* has only one species i.e. *papaya*. They are found primarily in tropical regions of Central and South America and Africa. They are typically short-lived evergreen shrubs or small trees growing to 5–10 m tall [1] *Carica papaya* L. (pawpaw), is an herbaceous plant belonging to the family Caricaceae and is commonly known as papaya. It is distributed throughout tropics and subtropics where it is extensively cultivated. It is a tropical fruit and is available throughout the year. It is much favoured and prized fruit all over the world for its flavor and nutritional properties. The whole plant is rich in an array of pharmacologically active secondary metabolites. All parts of papaya are therapeutically used. They show antimicrobial, antifungal, anti-amoebic, anti-fertility, anti-inflamatory, antioxidant, anti-tumor, anthelmintic, anti-hypertensive, anti-malarial, anti-ulcer, anti-sickling diuretic, hepatoprotective, histaminergic, hypoglycemic, immuno-modulatory, wound healing activity [2]. Some of the activities shown by different parts is given in Fig. 1.

It is apparent that *Carica papaya* is very important and useful plant. It becomes necessary to lay down quality control parameters and standardization is very necessary to prevent it from adulteration and help in maintaining its therapeutic efficacy. Once the plant is dried and converted to powder, paste or ointment as drug form, it loses its morphological identity and is easily prone to adulteration and substitution. Adulteration and substitution is directly proportional to the efficacy of the plant. Inspite of much technological advancement, pharmacognostic studies still hold upright position since they are simple, easy and economic. Pharmacognostic studies does not require any sophisticated equipment or training.

Pharmacognostic studies involve organoleptic, macroscopic, microscopic and powder studies, physicochemical studies, phytochemical studies, fluorescence studies, etc. They have to be attempted for the plant under study before it can be taken up for drug formulations.

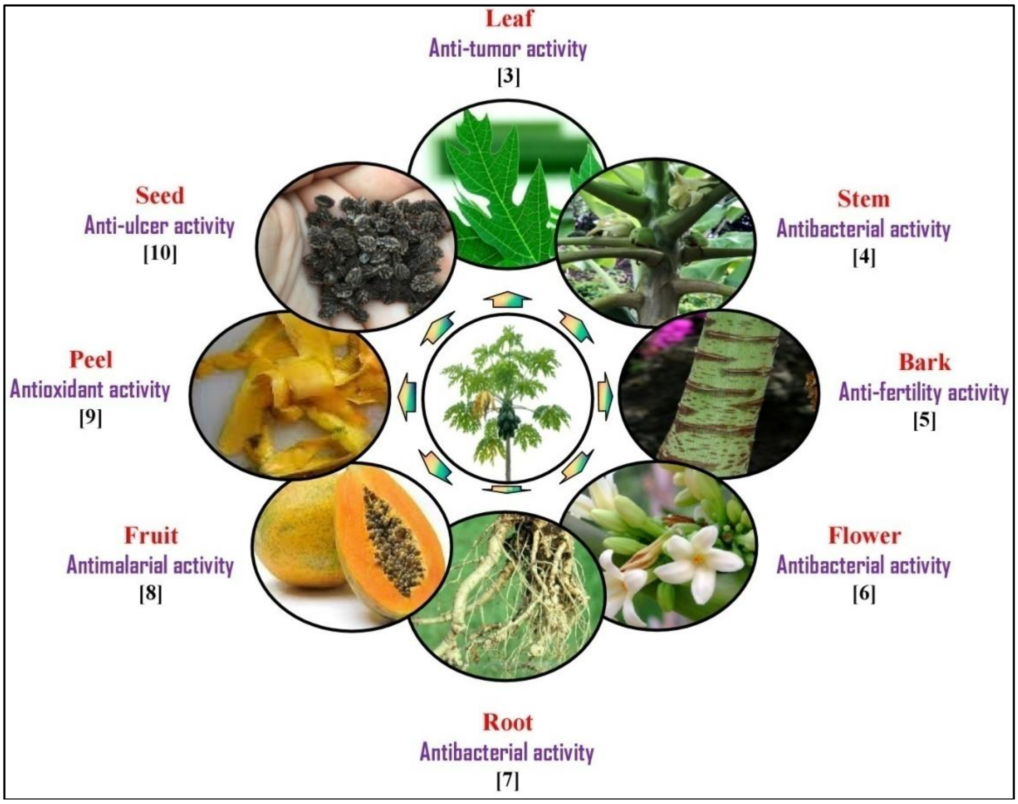


Fig 1: Reported biological activities of different parts of *Carica papaya* L.

The fruit of *Carica papaya* is eaten both ripe and un-ripe but its seeds are thrown away. Generally vegetable and fruit peels, seeds, etc are thrown away into the environment but there are reports that this waste is rich in many secondary metabolites and they can be therapeutically used. This will reduce environmental and agro waste disposal problems. In traditional system of medicine, like all other parts of papaya, seeds are also used to cure a number of ailments. Hence, it was thought of interest to lay down quality control parameters of un-ripe and ripe seeds of papaya. In this paper, pharmacognostic evaluation of ripe and un-ripe seeds of papaya is attempted. Macroscopic, microscopic and powder features, qualitative phytochemical, physicochemical properties and fluorescence characteristics were determined in both ripe and un-ripe seeds using standard methods.

2. Material and Methods

2.1 Plant collection

The un-ripe and ripe fruits of *Carica papaya* L. were purchased in 2018 from Rajkot, Gujarat, India. The un-ripe and ripe seeds were separated from fruit, washed thoroughly with tap water, shade dried and homogenized to fine powder and stored in closed containers for further studies.

2.2 Pharmacognostic study

Macroscopic, microscopic and powder microscopy study

The seeds of *C. papaya* were subjected to macroscopic studies as described by Khandelwal [11]. The parameters evaluated were the arrangement, size, shape, base, margin, apex, colour, odour, taste. For microscopic evaluation of seeds, thin transverse sections were made. They were washed with water and mounted in glycerine for observation and confirm its lignifications (10x, 40x) [12]. The powder microscopy of dried powder of seeds was studied using standard procedures [13]. The characteristic features observed were recorded by taking their photographs.

2.3 Physicochemical analysis

The physic chemical parameters like loss on drying, total ash, acid-insoluble ash, water-soluble ash, sulphated ash, nitrated ash and carbonated ash and extractive values in solvents of different polarity were determined in both un-ripe and ripe seeds of *C. papaya* as per WHO guidelines [14]. The procedure followed is as described earlier [15].

2.4 Qualitative phytochemical analysis

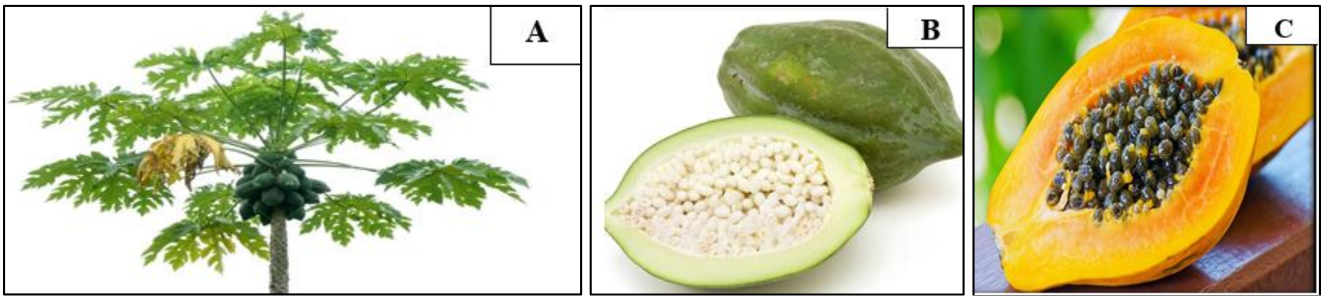
Qualitative analysis for the detection of phytoconstituents in un-ripe and ripe seeds powder was carried out following the procedure of Harborne [16]. Alkaloids were detected by using three reagents viz. Dragondroff's reagent, Mayer's reagent and Wagner's reagent separately. The tests were scored positive on the basis of orange precipitate, creamish precipitates and brown precipitate respectively. Phenols were detected by ferric chloride reagent. The crude powder of un-ripe and ripe seeds was treated with a few drops 5% ferric chloride solution. Formation of deep blue colour indicated the presence of phenols. Flavonoids were detected by alkaline reagent test. The crude powder of un-ripe and ripe seeds was treated with a few drops of diluted sodium hydroxide. Formation of intense yellow colour which turned colourless on addition of few drops of diluted HCl indicated the presence of flavonoids. Saponins were determined by Frothing test. The formation of stable froth upon vigorous shaking of crude powder of un-ripe and ripe seeds with distilled water indicated a positive test. Cardiac glycosides were detected by Keller-Kiliani test. The crude powder of un-ripe and ripe seeds was treated with 5% $FeCl_3$ and glacial acetic reagent to which few drops of concentrated H_2SO_4 was added. Appearance of greenish blue colour within few minutes indicated the presence of cardiac glycosides. Tannins were detected by $FeCl_3$ test. The crude powder of un-ripe and ripe seeds was treated with alcoholic ferric chloride ($FeCl_3$) reagent. Appearance of blue colour indicated the presence of tannins. Triterpenes were detected by the addition of concentrated H_2SO_4 to the chloroform extract of crude powder of un-ripe and ripe seeds. Appearance of reddish brown ring indicated the presence of triterpenes. Steroids were detected by Liebennann-Burchard test. The chloroformic solution of the crude powder of un-ripe and ripe seeds was treated with acetic anhydride and a few drops of concentrated H_2SO_4.Appearance of blue green ring indicated the presence of steroids. Phlobatannis were detected by boiling the crude powder of un-ripe and ripe seeds with aqueous HCl. Formation of red precipitate indicated the presence of phlobatannis. Coumarins were detected by the appearance of yellow colour on treatment of crude powder of un-ripe and ripe seeds with 10% NaOH. Leucoanthocyanins were detected by treating the crude powder of un-ripe and ripe seeds with isoamyl alcohol in equal proportion. Appearance of red colour in upper layer indicated the presence of leucoanthocyanins. Anthocyanins were detected by the appearance of blue colour on treatment of crude powder of un-ripe and ripe seeds with NaOH. Quinones were detected by addition of conc. HCl to crude powder of un-ripe and ripe seeds. Formation of yellow precipitation indicated the presence of quinones.

2.5 Fluorescence Analysis
Fluorescence study of crude powder of un-ripe and ripe seeds was performed as per Chase and Pratt [17]. A small quantity of the dry powder of un-ripe and ripe seeds was placed on grease free clean microscopic slide and 1 - 2 drops of freshly prepared reagent solutions were added, mixed by gentle tilting of the slide and waited for a few minutes. Then the slide was placed inside the UV chamber and observed in visible light, short (254 nm) and long (365 nm) ultra violet radiations. The colours observed by application of different reagents in different radiations were recorded.

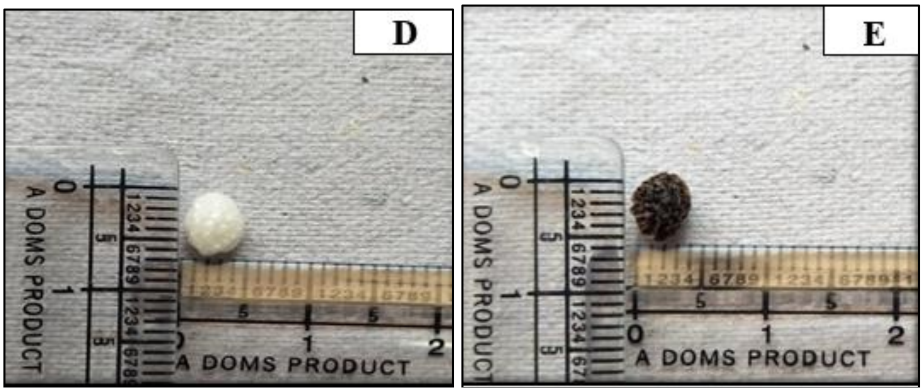
3. Results
3.1 Organoleptic and macroscopic characteristics
Organoleptic and macroscopic characteristics of *C. papaya* un-ripe and ripe seeds is given in Table 1 and Fig. 2. The macroscopic study showed that un-ripe and ripe seeds were of clavate shape. The size of un-ripe seed size was 0.7 cm long and 0.4 cm wide while that of ripe seed was 0.6 cm long and 0.4 cm wide. In both the seeds, the hilum was of wavy type. The unripe seed was creamiest white in colour with characteristics odour and bitter in taste. The ripe seed was dark black in colour with characteristics odour and salty in taste. The un-ripe and ripe seeds were smooth with wavy margin.

Table 1: Organoleptic features of *Carica papaya* un-ripe and ripe seed

Parts	Observation	
	Un-Ripe	**Ripe**
Part	Seed	Seed
Hilum	Wavy type	Wavy type
Size	0.7 cm long, 0.4 cm wide	0.6 cm long, 0.4 cm wide
Shape	Clavate	Clavate
Color	Creamiest white	Dark black
Order	Characteristic	Characteristic
Taste	Bitter	Salty
Appearance	Smooth	Smooth
Placentation	Parietal	Parietal
Apex	Acuminate	Acuminate
Base	Symmitrycal	Symmitrycal
Pedicel	Sessile	Sessile
Texture	Irregular smooth	Irregular smooth



Carica papaya* tree** **Un-ripe seed of *C. papaya **Ripe seed of *C. papaya***



Un-ripe seed **Ripe seed**

Fig 2: Macroscopic characteristics of *Carica papaya* L. un-ripe and ripe seed

3.2 Microscopic characteristics
The transverse section of *C. papaya* ripe seed is shown in Fig. 3. The T.S of seed was 47/7pb 5r 7divided into four parts epicarp, mesocarp, testa and endocarp. The epicarp was singe layered and was surrounded by thin smooth cuticle layer (Fig. 3A). The epicarp cells were polygonal parenchymatous type; testa was 3-4 layered, thick walled with cellulosic parenchymatous cells. Endocarp consisted of sclerenchyma cells with oil globules (Fig. 3B). The plasmodesma was yellowish in colour, and consisted of radially elongated thick walled mucilaginous cells (Fig. 3C). The upper surface of endocarp was single layered, consisted of polygonal shape parenchymatous tissue (Fig. 3D). The endocarp was thick walled with cellulosic sclrenchymatous cells, consisted of pitted aleuronic grain cells, oil globules and dicotyle structure (Fig. 3E). Several square crystals of calcium oxalate were present in endocarpic cells (Fig. 3F). All the characters were same in un-ripe seed.

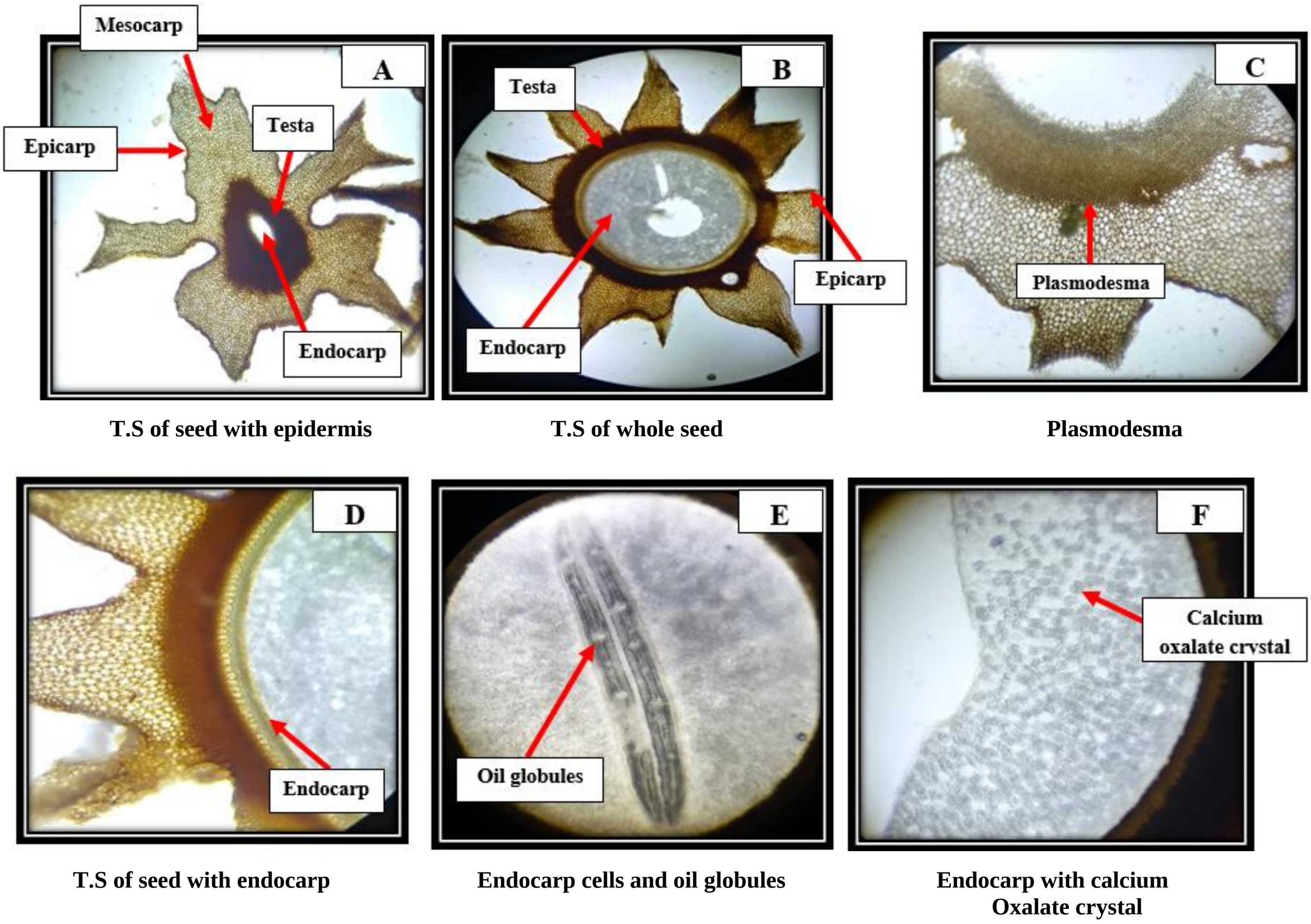
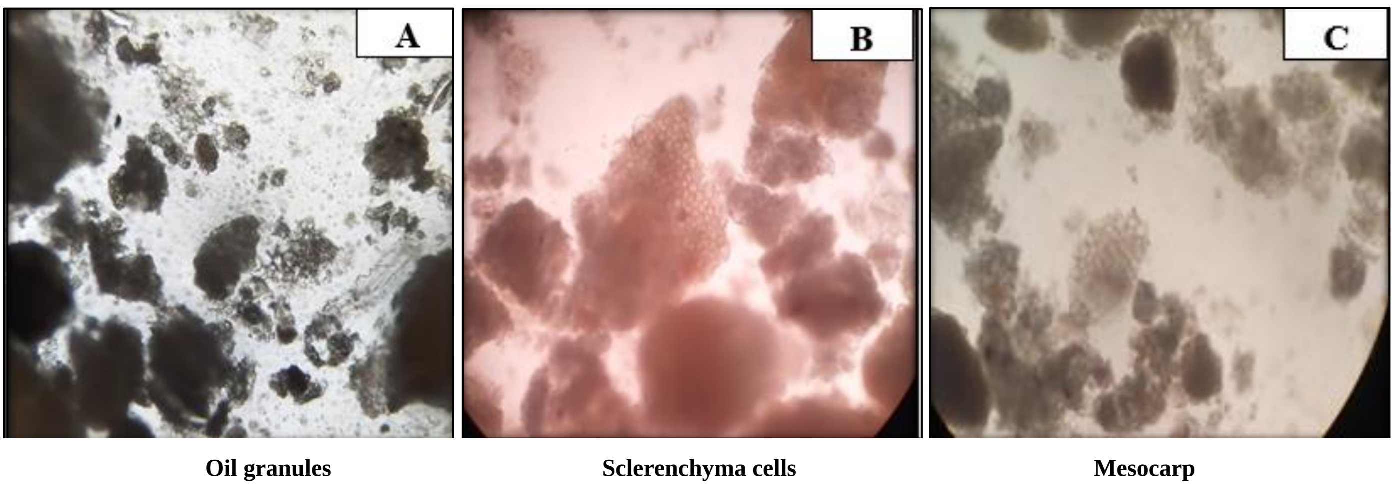
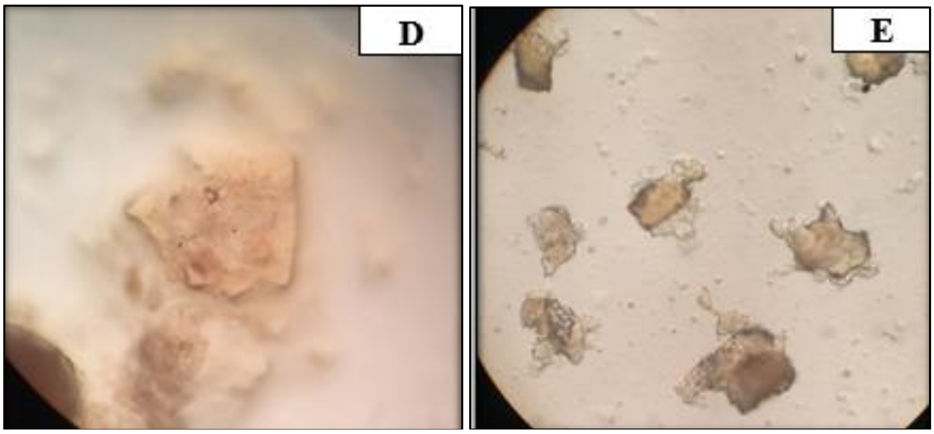


Fig 3: Microscopic characteristics of *C. papaya* ripe seed

3.3 Powder microscopic study
The crude powder of *C. papaya* un-ripe seed was creamiest white in colour, fine, odour was characteristics and taste was bitter. The specific characteristics determined from the powder study under microscopic investigation showed oil granules, sclerenchyma cells, mesocarp, square crystals of calcium oxalate and vittae (Fig. 4).

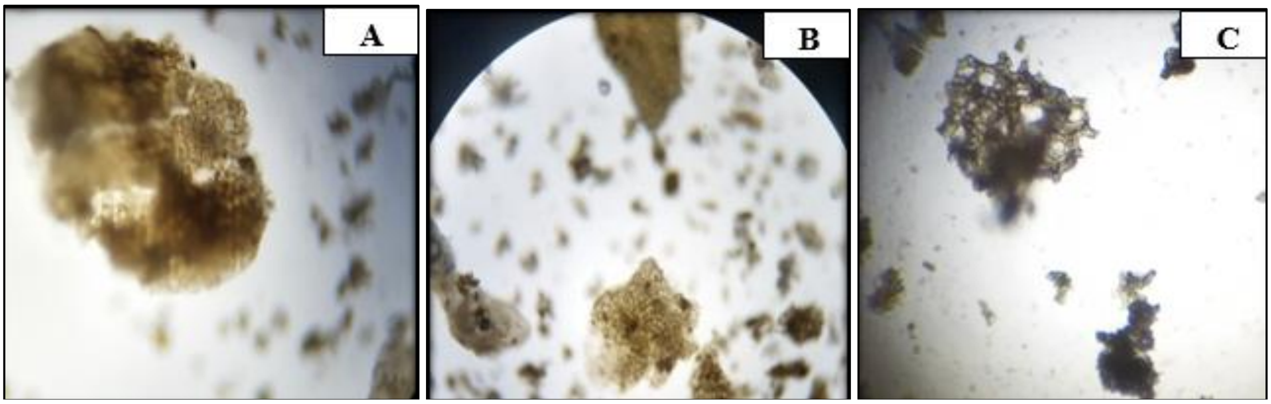




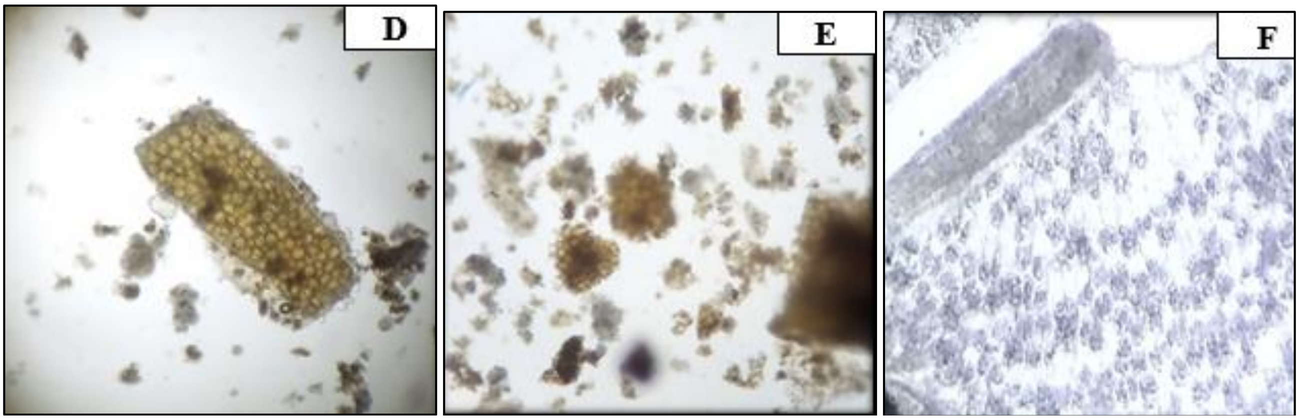
Square crystal of calcium oxalate crystals **Vittae**

Fig 4: Microscopic characteristics of powder of *C. papaya* un-ripe seed

The crude powder of *C. papaya* ripe seed was dark black in colour, fine, odour was characteristics and taste was salty. The specific characteristics determined from the powder study under microscopic investigation showed sclerenchyma cells, oil granules, mesocarp, endosperm with aleurone grains, vittae and square crystals of calcium oxalate (Fig. 5).



Sclerenchyma cells **Oil granules** **Mesocarp**



Endosperm with Aleurone grains **Vittae** **Square crystal of calcium oxalate crystals**

Fig 5: Microscopic characteristics of powder of *C. papaya* ripe seed

3.4 Physicochemical analysis

The physicochemical analysis of *C. papaya* un-ripe and ripe is given in Table 2. The loss on drying of un-ripe seed powder was 11.5% while that of ripe seed powder was 13.33%. The total ash, water soluble ash and acid insoluble ash of ripe seed was slightly less than that of un-ripe seed. The total ash of ripe seed was about 10 % while that of un-ripe seed was about 6%. In both the seeds, the water soluble ash was more than acid insoluble ash. All the three ashes (Sulphated ash, nitrated ash and carbonated ash) were slightly less in ripe seed than in un-ripe seed. The ashes were in the range of 9 to 15 %. In both the seeds, the extractive value was minimum in hexane and maximum in toluene. Almost all the extractive values of un-ripe seed were more than that of ripe seed. The water solubility of unripe seed was more than alcohol solubility while the water solubility of ripe seed was very less when compared to that of alcohol solubility.

Table 2: Physiochemical analysis of *C. papaya* un-ripe and ripe seed

No	Parameters	%Value (w/w) seed	
		Un-ripe	Ripe
1	Loss on drying	11.5	13.33
2	Total ash	9.75	6.42
3	Water soluble ash	3.33	2.33
4	Acid insoluble ash	0.83	0.67
5	Sulphated ash	14.5	10.5
6	Nitrated ash	10.67	9.83
7	Carbonated ash	11.83	11.67
8	Extractive value -HE	9.42	5.63
9	Extractive value -TO	19.89	16.21
10	Extractive value -EA	16.2	11.91
11	Extractive value -AC	13.45	15.96
12	Extractive value -ME	15.99	6.32
13	Extractive value -AQ	29.68	3.38

(HE-Hexane, TO-Toluene, EA-Ethyl acetate, AC-Acetone, ME-Methanol, AQ-Water)

3.5 Qualitative phytochemical analysis of crude powder

The qualitative phytochemical analysis of the crude powder of *C. papaya* un-ripe and ripe seeds is given in Table 3. In un-ripe seed, alkaloids, phenols and saponins were present in maximum amount; tannins, coumarins and leucoanthocyanins were present in moderate amount while triterpenes and steroids were present in trace amount; other phytoconstituents were absent. In ripe seed, alkaloids, triterpenes and saponins were present in moderate amount and none of the other phytoconstituents were detected.

Table 3: Qualitative phytochemical analysis of *C. papaya* un-ripe and ripe seed

No.	Phytochemical	Seeds	
		Un-ripe	Ripe
1	Alkaloids		
	i) Mayer's Reagent	+++	++
	ii) Wagner's Reagent	++	+++
	iii) Dragondroff's Reagent	++	-
2	Phenols	+++	-
3	Flavonoids	-	-
4	Saponins	+++	++
5	Cardiac glycosides	-	-
6	Tannins	++	-
7	Steroids	+	-
8	Triterpenes	+	++
9	Phlobatannis	-	-
10	Coumarins	++	-
11	Leucoanthocyanins	++	-
12	Anthocyanins	-	-
13	Quinones	-	-

(+++) - High amount, (++) - Moderate amount, (+) - Less amount, (-) – Absent

3.6 Qualitative phytochemical analysis of different solvent extracts

Qualitative phytochemical analysis of different solvent extracts of un-ripe seeds is given in Table 4. None of the phytoconstituents were detected in hexane, toluene, ethyl acetate and acetone solvent extracts. In methanol extract, phenols, cardiac glycosides and tannins were present in high amount while flavonoids, saponins, triterpenes, steroids and quinones were present in trace amount.In aqueous extract, almost all the phytoconstituents detected were in moderate amount. The phytoconstituents detected were alkaloids, phenols, flavonoids, cardiac glycosides, quinones, tannins and triterpenes.

Table 4: Qualitative phytochemical analysis of *C. papaya* L. un-ripe seed

No.	Phytochemical	HE	TO	EA	AC	ME	AQ
1	Alkaloids						
	i) Mayer's Reagent	-	-	-	-	-	++
	ii) Wagner's Reagent	+	+	+	+	++	+++
	iii) Dragondroff's Reagent	+	+	+	+	-	-
2	Phenols	-	-	-	-	+++	++
3	Flavonoids	-	-	-	-	+	++
4	Saponins	-	-	-	-	+	-
5	Cardiac glycosides	-	-	-	-	+++	++
6	Tannins	-	-	-	-	+++	++
7	Steroids	+++	++	+++	+++	+	-
8	Triterpenes	+	-	-	+	+	++
9	Phlobatannis	-	-	-	-	-	-
10	Coumarins	-	-	-	-	-	+
11	Leucoanthocyanins	-	-	-	-	-	-
12	Anthocyanins	-	-	-	-	-	-
13	Quinones	-	-	-	-	+	++

Note: (+++) - High amount, (++) -Moderate amount, (+) -Less amount, (-) –Absent

Qualitative phytochemical analysis of different solvent extracts of ripe seeds is given in Table 5. In ripe seed, alkaloids and steroids were present in moderate amount in all the solvent extracts. In methanol extract, trace amount of few phytoconstituents were present. On the other hand, in aqueous extract almost all the phytoconstituents were detected; alkaloids, phenols, cardiac glycosides and tannins were maximum; while flavonoids, phlobatannis, coumarins, quinones were present in moderate amount. Other phyto constituents were found in trace amount. Saponins and steroids were absent.

Table 5: Qualitative phytochemical analysis of *C. papaya* L. ripe seed

No.	Phytochemical	HE	TO	EA	AC	ME	AQ
1	Alkaloids						
	i) Mayer's Reagent	-	-	-	-	+	+++
	ii) Wagner's Reagent	++	++	++	++	+++	+++
	iii) Dragondroff's Reagent	+	+	+	+	+	+++
2	Phenols	-	-	-	-	-	+++
3	Flavonoids	-	-	-	-	+	++
4	Saponins	-	-	-	-	-	-
5	Cardiac glycosides	-	-	-	-	-	+++
6	Tannins	-	-	-	-	-	+++
7	Steroids	++	++	++	++	+++	-
8	Triterpenes	-	-	-	-	+	+
9	Phlobatannis	-	-	-	-	+	++
10	Coumarins	-	-	-	-	+	++
11	Leucoanthocyanins	-	-	-	-	-	+
12	Anthocyanins	-	-	-	-	-	+
13	Quinones	-	-	-	-	+	++

Note: (+++) - High amount, (++) -Moderate amount, (+) -Less amount, (-) –Absent

3.7 Fluorescence analysis

The fluorescence characteristics of un-ripe and ripe seeds powder of *C. papaya* are summarized in Table 6. *C. papaya* un-ripe and ripe seed powder was treated with a number of different reagents, which showed characteristic fluorescence. The powder of un-ripe and ripe seeds showed different colors in visible light, short and long ultra violet radiations. The colors observed were brown, brownish yellow, yellowish green, red, green, dark yellow, dark brown while that observed for ripe seeds were black, dark green, red.

Table 6: Fluorescence analysis of *C. papaya* L. un-ripe and ripe seeds powder

Seeds Powder	Visible light	Under UV light Short Wavelength (254 nm)	Long Wavelength (365 nm)
Un-ripe	Brown	Black	Greenish black
Ripe	Black	Black	Black
Powder + 1 N NaOH (aq)			
Un-ripe	Brown	Black	Green
Ripe	Black	Black	Black
Powder + 1 N NaOH (alco)			
Un-ripe	Brown	Greenish black	Light green
Ripe	Dark green	Black	Blackish green
Powder + Ammonia			
Un-ripe	Brownish Yellow	Black	Yellow
Ripe	Black	Black	Black
Powder + Picric acid			
Un-ripe	Yellowish green	Black	Green
Ripe	Dark green	Black	Black
Powder + Petroleum ether			
Un-ripe	Brownish Yellow	Black	Yellow
Ripe	Dark green	Black	Black
Powder + 50% HCl			
Un-ripe	Brown	Black	Green
Ripe	Dark green	Black	Black
Powder + 50% H_2SO_4			
Un-ripe	Red	Black	Light green
Ripe	Dark green	Black	Black
Powder + Ethyl acetate			
Un-ripe	Brown	Black	Yellowish green
Ripe	Dark green	Black	Black
Powder + Ethyl alcohol			
Un-ripe	Green	Light black	White
Ripe	Dark green	Black	Dark green
Powder + Methanol			
Un-ripe	Brown	Black	Light green
Ripe	Dark green	Black	Black
Powder + 50% KOH			
Un-ripe	Brown	Black	Dark green
Ripe	Black	Black	Black
Powder + 50% HNO_3			
Un-ripe	Red	Black	Black
Ripe	Red	Black	Black
Powder + Acetic acid			
Un-ripe	Brown	Black	Light green
Ripe	Black	Black	Black
Powder + Iodine in Water (1%)			
Un-ripe	Dark yellow	Dark green	Light green
Ripe	Dark green	Black	Dark green
Powder + $FeCl_3$			
Un-ripe	Dark brown	Black	Green
Ripe	Dark green	Black	Black

4. Discussion

In order to maintain the therapeutic efficacy of natural drugs, it is very essential to lay down quality control and standardization parameters. Pharmacognostic studies are thus very important and parameters established in such studies can act as reference standards and identity of that particular plant and part. The quality, purity and authenticity of the drug can be maintained which in turn will help to maintain its efficacy. Such studies are attempted for herbal drugs involving different parts of the plant for eg. Flower [18]; root [19]; stem, root, leaf [20], leaf and stem [21]; fruit [22]; stem bark [23] peel [24], etc.

C. papaya is a versatile tree and all the parts are very useful in the treatment of various diseases. The pharmacognostic studies will help in establishing its botanical identity. The organoleptic, macro and microscopic characteristics and powder study revealed the specific characters that are the diagnostic characters of this plant. It will help to identify the plant when intact or when in powder form. These parameters will help to correctly identify the plant and prevent it from being adulterated. Various parameters evaluated in physicochemical analysis reveal the nature and quality of plant powder (drug) under study. Loss on drying value reveals the moisture content of the sample and whether it is properly dried or not. If it is high, it will facilitate the growth of microorganisms and might render the sample to decay. The ash values reveal the inorganic elements and other impurities like silica, carbonates, nitrates, sulphates present in the drug and extractive values reveal the nature of phytochemicals present and their solubility or insolubility in a particular solvent. The phytochemical analysis reveals their presence and solubility in a particular solvent. The seed powder was rich in alkaloiads, tannins, steroid, phenols and cardiac glycosides. Hence it can be successfully used as a natural source for anticancer, antioxidant, antibacterial, anti-inflammatory agents [25-27]. The fluorescence analysis is also characteristic of a particular plant. Its fluorescence will change if it is in impure form. Such pharmacognistic studies are reported for other plants [28-31]. Pharmacognostic study of leaves of *C. papaya* has been reported by Zunjar *et al.* [32], Verma and Vaidya [33] and Priyadarshi and Ram [34] while for seed there is only one report of few parameters of pharmacognostic study [35]. We are perhaps the first one to do pharmacognostic studies of un-ripe and ripe seed of *C. papaya*. Generally pharmacognostic studies are reported for leaves but few studies have been done on seeds for eg. *Mimusops elengi* [36], *Bambusa arundinacea* [37], *Cassia occidentalis* [38], *Aegle marmelos* [39].

In conclusion, it can be stated that the parameters evaluated in this study can act as reference standards of *C. papaya* un-ripe and ripe seeds. They are the diagnostic features of the seeds and can be useful in preparation of monograph. They will help in maintaining the identity, authenticity and purity of this important versatile medicinal plant. The information generated in this work will work as standard and help to identify the adulterants and impurities. The results of photochemical studies will help the researchers to choose proper solvent for drug formulations. All the parameters evaluated and standards laid down are enough to identify the genuine drug from adulterated drug.

5. Acknowledgments

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