

CHEMICAL CHARACTERIZATION OF THE ACTIVE CONSTITUENTS PRESENT IN DIFFERENT FORMS OF *EMBLICA OFFICINALIS* (AMLA)

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ABSTRACT

The chemical characteristics of different forms of *Emblica officinalis* - fresh amla, sweet amla, dried amla, salted amla and lehyam - were analyzed for total phenol, vitamin C, carbohydrate, fat and total antioxidant capacity. The ash of the respective samples was used to determine the iron, calcium and phosphorus content. Higher percentage of total antioxidant capacity was observed in all the samples, which depends on the concentration of the phenolic compounds. In conclusion it can be stated that the vitamin C content (361.90mg/100g) and iron content (0.012mg/100mg) were higher in fresh form of *Emblica officinalis* whereas dried amla showed higher level of phosphorus (0.14%), phenolic acids (10%) compared to all other form of *Emblica officinalis*.

Key-words: Chemical characteristics, *Emblica officinalis*, total phenol, vitamin C.

1. INTRODUCTION

Amla (Figure 1) belonging to Euphorbiaceae family known as *Phyllanthus emblica*, Indian gooseberry or *Emblica officinalis* is a natural, efficacious, antioxidant, with the richest natural source of vitamin C. Numerous studies conducted on *Emblica officinalis* fruit suggest that it has anti-viral properties and also functions as an anti-bacterial and anti-fungal agent (Sampath Kumar *et al.*, 2012). According to ancient Indian, it is the first tree to be created in the Universe. The species is native to India and also grows in tropical and subtropical regions including Pakistan, Uzbekistan, Srilanka, South East Asia, China and Malaysia. The fruits of *Emblica officinalis* are widely used in the Ayurveda and are believed to increase defense against diseases. It has its beneficial role in cancer, diabetics, liver treatment, heart trouble, ulcer, anemia and various other diseases (Sachan *et al.*, 2013). Similarly, it has application as antioxidant, immunomodulatory, antipyretic, analgesic, cytoprotective, antitussive and gastro protective agent. Additionally, it is useful in memory enhancing, ophthalmic disorders and lowering cholesterol level. It is believed by ayurvedic practitioners that regular intake of amla reduces the onset of chronic diseases (Khan, 2009).

The edible fruit tissue contains protein concentration 3-fold and ascorbic acid concentration 160-fold compared to that of the apple (Puri *et al.*, 1970). Fruits, whether fresh or dried, have always formed a part of the stable diet of human beings. The reason for that, they are rich in nutrients and provide some of the essential minerals, vitamins and the like, to our body. Apart from that they also help

in curing a number of diseases, *Emblica officinalis* is one of the important herbal drugs used traditionally both as a medicine and as tonic to build up, lost vitality and vigor.

Figure 1. Amla - *Emblica officinalis*



No published data could be found relative to the composition of the various forms of amla, hence in the present study the chemical constituents in the different forms of *Emblica officinalis* like fresh amla, sweet amla, dried amla, salted amla and lehyam was studied.

2. MATERIALS AND METHODS

2.1. Preparation of samples

5g of fresh amla (Figure 2), sweet amla (Figure 3), dried amla (Figure 4), salted amla (Figure 5) and amla lehyam (Figure 6) were ground with 50ml of water by using motor pestle. These solutions were filtered and the filtrates were stored in different containers.

2.2. Determination of total phenols

0.1ml of each samples were taken in a different test tubes. 0.9ml of distilled water and 0.5ml of Folin-Ciocalteu reagents were added

(Singleton and Rossi, 1965). After three minutes, 2ml of 20% sodium carbonate was added to it. Likewise, the blank was also prepared. The test tubes were heated for one minute and cooled. Then, the optical densities were measured at 650nm using UV-Spectrophotometer.



Figure 2 Fresh amla Figure 3 Sweet amla

Figure 4 Dried amla



Figure 5 Salted amla



Figure 6 Lehyam



10, 20, 30, 40 and 50ml of standard gallic acid solutions were prepared and optical densities were noted. The graph was plotted absorbance against concentration. The concentration of phenols in the aliquot of the samples can be read directly from the calibration.

2.3. Determination of Vitamin C

10ml of each sample were taken in a separate 100ml conical flask and titrated with the dye solution. The end point was the appearance of pink colour (Sadasivam *et al.*, 1987).

$$\text{Ascorbic acid} = \frac{\text{Titre value} \times \text{Dye factor} \times \text{Volume made up} \times 10}{\text{Aliquot of extract taken for estimation} \times \text{Volume of the sample}}$$

2.4. Determination of Carbohydrates

01 g of dried amla and 1ml of fresh amla, sweet amla, salted amla and lehyam extracts were taken in separate boiling test tubes. These samples were digested with 10ml 2.5N hydrochloric acid for three hours. After digestion, the solutions were neutralized with sodium carbonate. Then, the solutions were filtered and made up to 100ml with water. 0.5ml of samples was taken in separate test tubes. 0.9ml of distilled water and 4ml of Anthrone reagent were added to it. The blank solution was also prepared. The test tubes were boiled for 10 minutes and cooled. Then, the optical densities were measured at 630nm using UV-Spectrophotometer. 10, 20, 30, 40, 50, 60, 80, 100ml of standard glucose solutions were prepared and optical densities were noted using UV-Spectrophotometer. The graph was plotted with absorbance against concentration. The concentration of carbohydrates in the aliquot of samples can be read directly from the calibration (Hedge *et al.*, 1962)

2.5. Determination of Iron

1g of each samples were ashed in separate crucibles. The ashes were digested with 2ml of Conc. HCl, 2ml of picric acid and 2ml of Conc. HNO₃. After digestion, the solutions were diluted to 10ml with distilled water. 1ml of the each diluted solutions were taken in separate test tubes. 0.5ml of Conc. H₂SO₄, 1ml of potassium persulphate and 2ml of potassium thiosulphate were added to it and made up to 15ml with distilled water. The blank was also prepared. Then, the optical densities were measured at 480nm using UV-Spectrophotometer .

$$\text{Iron (mg/100g)} = \frac{\text{OD of sample} \times 0.1 \times \text{Total volume of ash solution} \times 100}{\text{OD of standard} \times 5 \times \text{weight of sample taken for ashing}}$$

02 , 0.4, 0.6, 0.8, 1ml of standard iron sulphate solutions was prepared and optical densities of solutions were measured. The graph was plotted with absorbance against concentration. The concentration of iron contents in the aliquot of the samples can be read directly from the graph.

2.6. Determination of Calcium

1g of each samples were ashed in separate crucibles. The ashes were digested with 2ml of Conc. HCl, 2ml of picric acid and 2ml of Conc. HNO₃. After digestion, the solutions were diluted to 10ml with distilled water. 0.5ml of the diluted solutions were taken and made up to 10ml with distilled water. The standard solution was prepared. Then, the amount of calcium present in the samples was measured by using flame photometer.

2.7. Determination of Fat

10ml of samples were refluxed with 20ml of hexane for one hour. Then, the solutions were filtered off. The filtrates were collected in separate previously weighed petri plates. Again 20ml of hexane was added to the reflux container. The solutions were refluxed and filtered. The filtrates were combined and kept in the oven. The contents of the petri plates were dried and weighed. It gave the amount of fat present in the samples (Sadasivam *et al.*, 1987).

2.8. Determination of Phosphorus

01 ml acid digestion solutions of samples were taken in separate 100ml standard flasks and made up to 100ml with distilled water. One drop of phenolphthalein indicator was added to it. The pink was obtained. Then, dilute sulphuric acid was added in drop wise to discharge the colour. 2ml of ammonium molybdate and 0.25ml of stannous chloride were added to it. The solutions were mixed thoroughly. The blank solution was also prepared. After 10 minutes, the optical densities of the solutions were measured at 690nm using UV-Spectrophotometer.

5, 10, 15, 20, 25ml of standard potassium dihydro phosphate solutions were prepared and optical densities of the solutions were measured. The graph was plotted with absorbance against concentration. The concentration of phosphorus in the aliquot sample can be read directly from calibration.

2.9. Determination of Total antioxidant capacity

02 ml of each sample extracts were taken in separate test tubes. 0.2ml of 2.5% linoleic acid, 0.4ml of phosphate buffer solution (pH=7) and 0.2ml distilled water were added to it. The test tubes were kept at dark in 40°C for five hours. 0.1ml of the solutions was taken for analysis. 9.7ml of 75% ethanol and 0.1ml of 30% ammonium thiocyanate were added and left for five minutes. 0.1ml 20mM FeCl₃ in 3.5% HCl was added to it. The blank and control solutions were also prepared. Then, the

optical densities of the samples were measured at 500nm using UV-Spectrophotometer. Total antioxidant capacity is then calculated using following formula.

$$\text{Total antioxidant capacity} = \frac{\text{Control value} - \text{OD value} \times 100}{\text{Control value}}$$

3. RESULTS AND DISCUSSION

Amla is one of the most celebrated herbs in the Indian traditional medicine system, and is becoming increasingly well known for its unusually high levels of Vitamin C, which is resistant to storage and heat damage due to cooking. Its fruits have potent antioxidant activity due to the presence of tannins, vitamin C and flavonoids. It has been used as rejuvenating herb.

In the present study, the chemical constituents of fresh amla fruit were compared with dried, lehyam, salted and sweetened preserved form of amla. The parameter that essentially determine the active constituents of the fruit was analyzed and it was observed that dried *Emblica officinalis* showed higher levels of chemical constituents compared to other forms of amla. The lehyam that contain high content of amla along with other plants, showed high level of fat, which could be due to the addition of ghee and oil during preparation of chayawaprash. The fat content was similar in all other preserved forms of *Emblica officinalis*.

3.1. Total phenolic content

Total phenolic content of amla extract was determined by colorimetric method. In this method phosphotungstic-phosphomolybdenum complex obtained as a blue chromophore was measured. Total phenolic contents present in different forms of amla calculated from concentrations of aliquot solutions, fresh amla (15µg), dried amla (9µg), sweet amla (7µg), salted amla (2µg) and lehyam (14µg) showed varying concentrations of phenolic content (Graph 1). And they were having different amounts of phenolic contents in its extracts (Table 1).

In the present study total phenolic content was higher in dried amla (10%) compared to fresh amla (2.4%), sweet amla (1.23%), salted amla (0.344%) and lehyam (2.46%). Klimczak *et al.*, (2006) reported the content of total phenols in fresh orange juices to be 226.7±6.4mg/L (juice 1) and 202.7±6.8mg/L (juice 2). The protein content by Folin-Ciocalteu method was higher than the concentration obtained by HPLC method 684.2±1.0mg for juice 1 and 634.6±0.9mg of caffeic acid equivalents/L for juice 2.

The results are in good agreement with those reported in the literature (Rapisarda *et al.*, 1999; Gardner *et al.*, 2000) however there is evidence that the spectrophotometric method over estimates the poly-phenolic content as compared to the chromatographic method. This can be explained by the lack of selectivity, (Escarpa and Gonzalez, 2001), which reacts not only with phenols but also with other reducing compounds such as carotenoids, amino acids, sugar and vitamin C (Vinson *et al.*, 2001). However, this method has been shown to be a useful analytical tool for the routine analysis of polyphenols and it is widely used in many laboratories for the determination of differences among fruits and vegetables and their products.

3.2. Vitamin C

Vitamin C content of the amla extracts were calculated using dye factor. In this method ascorbic acid oxidized to give dehydroascorbic acid. Vitamin C contents present in different forms of amla represented in Table 1 indicate that vitamin C content was highest in fresh amla and lowest in salted amla.

Ranganna, (1986) reported that irrespective of the treatments and storage conditions, a continuous decrease in ascorbic acid content of amla juice was observed during storage. It was observed that after six months of storage, maximum vitamin C content (232.7mg/100ml) was observed in SO₂ treated juice stored at low temperature, followed by 195.5mg/100ml in pasteurized + SO₂ treated and 189.3mg/100ml in pasteurized amla juice stored at low temperature. In present study vitamin C content was higher in fresh amla (361.904mg/100g) compared to dried amla (222.63mg/100g), sweet amla (115.27mg/100g), salted amla lehyam (104.79mg/100g) and salted amla (40.97mg/100g). The significance of vitamin C as an important antioxidant has been well established (Miller and Rice-Evans, 1997; Rapisarda *et al.*, 1999; Gardner *et al.*, 2000). The concentration of vitamin C is a significant indicator of orange juice quality and it may serve as an indicator that all processes, which ensure a high quality of the product, have been applied in the production processes. In both juices analyzed in their study, the vitamin C content was found to be similar, 408.5±0.9mg/L and 361.5±1.8mg/L in juice 1 and 2 respectively. The author's previous study, where commercial oranges delivered by different producers were assessed, showed that they contain 150 to 440mg of vitamin C in 1L of juice.

3.3. Carbohydrates

Carbohydrate content of amla extract was determined by colorimetric method. In this method glucose is dehydrated to hydroxymethyl furfural. Carbohydrate contents present in different forms of amla calculated from concentrations of aliquot solutions. Fresh amla (10µg), dried amla (55µg), sweet amla (31µg), salted amla (8µg) and lehyam (21µg) showed wide variation in carbohydrate content (Graph 2). The results represented in Table 1 showed that the highest carbohydrate content was found in dried amla (10.4%).

Dhale, (2012) reported that reducing sugar in amla was 8.6%. In present study carbohydrate content was higher in dried amla (10.4%) compared to fresh amla (1.28%), sweet amla (0.272%), salted amla (0.068%) and lehyam (0.18%).

3.4. Iron

Iron content of amla extract was determined by colorimetric method. In this method ferric thiocyanate was obtained. The concentrations of the aliquot solutions were calculated from standard iron graph. Fresh amla (0.12µg), dried amla (0.6µg), sweet amla (0.11µg), salted amla (0.22µg) and lehyam (0.1µg) showed varying concentrations of iron content (Graph 3). Then, the iron contents of the different forms of amla were calculated (Table 1).

Mishra *et al.*, (2012) reported that iron content present in *Emblica officinalis* was 17.2ppm whereas Suriyavathana *et al.*, (2011) reported that iron content was present in *Emblica officinalis* was 0.606±0.0001mg/g. In the present study, iron content was higher in dried amla (0.06mg/100mg) compared to fresh amla (0.012mg/100mg), sweet amla (0.011mg/100mg), salted amla (0.0044mg/100mg) and lehyam (0.01mg/100mg).

3.5. Calcium

Calcium content of amla was determined by flame photometer using ash solution (Table 1) showed that the highest calcium content in salted amla (0.4%) compared to other forms of amla. Dhale, (2012) reported that calcium content in *Emblica officinalis* was 0.42%. Suriyavathana *et al.*, (2011) reported that calcium content was present in the *Emblica officinalis* was 4.804±0.0029mg/g. In present study, calcium content was higher in salted amla (0.4%) compared to fresh amla (0.3%), sweet amla (0.3%), dried amla (0.2%) and lehyam (0.2%).

3.6. Fat

Fat content of amla was determined by reflux method, varied between the different forms of

amla (Table 1). Fat content was very low in all samples compared to lehyam. Mishra *et al.*, (2012) reported that crude fat content present in *Emblica officinalis* was 2.46% whereas Sachan *et al.*, (2013) reported that fat content present in *Emblica officinalis* was 0.1%. In present study, fat content was higher in lehyam (2.6%) compared to fresh amla (0.8%), dried amla (2.2%), sweet amla (1.7%) and salted amla (0.8%).

3.7. Phosphorus

Phosphorus content of amla extract was determined by the formation of molybdenum blue complex. Phosphorus contents present in different forms of amla were calculated from concentrations of aliquot solutions. Fresh amla (3.5µg), dried amla (14µg), sweet amla (3µg), salted amla (5µg) and lehyam (4.5µg) showed varying concentrations of phosphorus content (Graph 4). Then, the phosphorus contents of the different forms of amla were calculated (Table 1).

Dhale, (2012) reported that phosphorus content present in *Emblica officinalis* was 0.04%. Suriyavathana *et al.*, (2011) reported that phosphorus content in *Emblica officinalis* was 0.716±0.0001mg/g. In present study, phosphorus content was higher in dried amla (0.14%) compared to fresh amla (0.035%), sweet amla (0.03%), salted amla (0.05%) and lehyam (0.045%).

3.8. Total antioxidant capacity

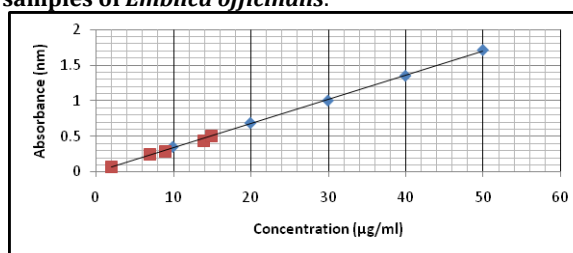
Total antioxidant capacities of the different forms of amla were calculated based on the formation of oxidized compounds in relation to the control (Table 1).

Ruangchakpe *et al.*, (2007) reported that total antioxidant capacity (µmoles TE/100g FW) in *Emblica officinalis* was 78.2 whereas Shukla *et al.*, (2009) reported that total antioxidant capacity in *Emblica officinalis* at concentration of 1mg/ml was 7.78±0.17. In present study, the total antioxidant capacity was higher in salted amla (52.55%) compared to fresh amla (49.5%), sweet amla (35.24%) and lehyam (32.58%). Maheshu *et al.*, (2011) reported that the ferric thiocyanate method determines the antioxidant activity with the measurement of the amount of peroxides formed in a linoleic acid emulsion of antioxidant, during incubation (Erkan *et al.*, 2008). The inhibitory effect of the extracts from Dolichos lablab raw and processed seed samples on the peroxidation of linoleic acid at concentration of 250µg/ml, in comparison to BHT was measured using the ferric thiocyanate method. Each extracts showed strong antioxidant activity in inhibition of linoleic acid

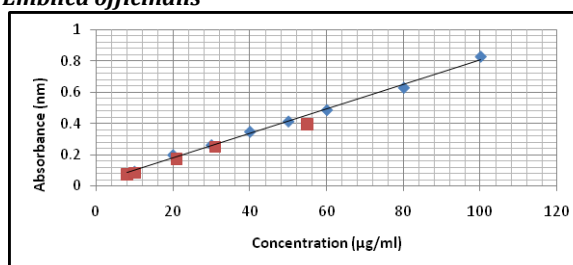
peroxidation. From the ferric thiocyanate results, the inhibition of peroxidation in linoleic acid system of raw, dry heated and pressure cooked samples were found to be 97.2±1.02%, 98.4±1.48% and 95.6±0.98%.

Antioxidant activities are known to increase proportionality to the polyphenol content. This activity is believed to be mainly due to their redox properties (Adedapo *et al.*, 2008), which plays an important role in (a) adsorbing and neutralizing free radicals, (b) quenching singlet and triplet oxygen and (c) decomposing peroxides (Wang *et al* 1998). Also according to recent reports, a highly positive relationship between total phenols and antioxidant activity appears to be the trend in many plant species. Naturally occurring antioxidants such as phenols, flavonoids are well known to have less or no side effects and hence are considered to safe (Asgarirad *et al.*, 2010).

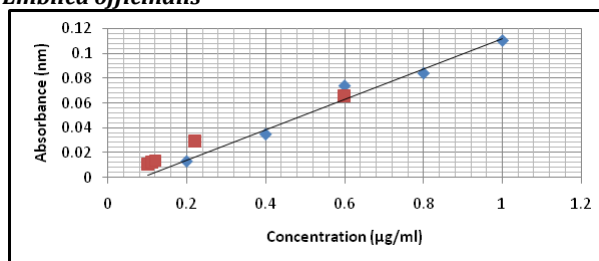
Graph 1. Standard graph of gallic acid used for the determination of total phenolic content in the test samples of *Emblica officinalis*.



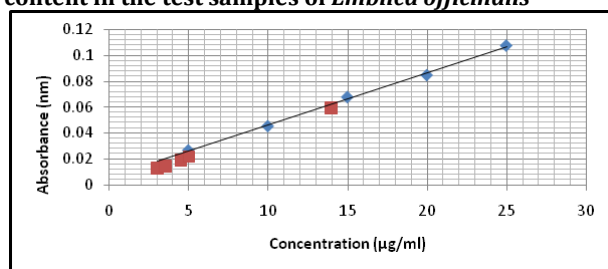
Graph 2. Standard graph of glucose used for the determination of carbohydrates in the test samples of *Emblica officinalis*



Graph 3. Standard graph of iron sulphate used for the determination iron content in the test samples of *Emblica officinalis*



Graph 4 Standard graph of potassium dihydro phosphate used for the determination of phosphorus content in the test samples of *Emblica officinalis*



4. CONCLUSION

The present study was carried out to assess the variations in the chemical characteristics of different forms of *Emblica officinalis*.

- All the samples were observed to be a good source of vitamin C and also antioxidant capacity.
- A correlation between phenolic compounds and antioxidant capacity was observed, and it can be concluded that the phenolic compounds contributes directly to antioxidant action.
- The vitamin C content (361.90mg/100g) was higher in fresh amla compared to other forms of *Emblica officinalis*.
- Fresh amla was showed high levels of total antioxidant capacity and vitamin C but lower levels of carbohydrate, fat and phosphorus content.
- Dried amla was high on total phenolic content, carbohydrate, iron, fat and phosphorus but showed low levels of vitamin C and iron compared to other forms of *Emblica officinalis*.
- All samples are found to have similar calcium content.
- Salted amla was high only on total antioxidant capacity (52.55%) but showed low levels of all other parameters
- Fresh amla and lehyam having similar total phenolic content and iron content but differ in other parameters.

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Table 1. Various chemical constituents in different forms of *Emblica officinalis*

Chemical Constituents	Fresh amla	Dried amla	Sweet amla	Salted amla	Lehyam
Total phenols	2.40	10.00	1.23	0.34	2.46
Vitamin C (mg/100g)	361.90	222.63	115.27	40.97	104.79
Carbohydrate (%)	1.28	10.40	0.27	0.06	0.18
Iron (mg/100mg)	0.012	0.060	0.011	0.004	0.010
Calcium (%)	0.30	0.20	0.30	0.40	0.20
Fat (%)	0.80	2.20	1.70	0.80	2.60
Phosphorus (%)	0.03	0.14	0.03	0.05	0.04
Total antioxidant capacity (%)	49.50	46.78	35.24	52.55	32.58