PROXIMATE AND ANTIOXIDATIVE CHANGES DURING MATURATION OF *DILLENIA PENTAGYNA* FRUIT

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Abstract
Enzymatic, non-enzymatic antioxidants and proximate value in the one plant of Dillenia pentagyna Roxb. of four time harvests stage during the maturation of fruits were studied. Our aims to pattern the antioxidant potential, amino acid profiling and nutrients in the fruit extracts were evaluated using Gas chromatography and Atomic absorption spectroscopy. The results revealed that the plant fruits contained appreciable amounts of proximate content was at the maximum level on the 55th days i.e. moisture, ash, crude fiber and malic acid content and then significantly decreased on the 10th day while, minerals i.e. sodium (Na), calcium (Ca), zinc (Zn) were increases of 27.1%, 41.11% and 65.9% while potassium and magnesium was decreased of 33.26% and 27.10% in the respect of 10th day fruit. The total phenol content in the fruit extracts (10 days, 25 days, 40 days and 55 days) expressed as gallic acid equivalent (GAE) was in the range of 0.352 to 0.703 mg GAE/g dw. Activity of Ascorbate peroxidase, guiacol peroxidase and glutathione reductase content in the fresh fruit extract (10D, 25D, 40D and 55D) expressed as µM/g fw were in the range of 406.90 to 708.66 µM/g fw, 0.372 to 0.207 µM/g fw and 0.117 to 0.184 µM/g fw while SOD, expressed as U/g fw was in the range 165.53 to 253.92 U/g fw. The results indicated that the Dillenia pentagyna fruits are nutrient-dense foods that can be good sources of ascorbic acid, α-Tocopherol as well as carbohydrates, dietary fiber and trace elements can be utilized as a natural source of antioxidant.

Key words: antioxidant activity, *Dillenia pentagyna*, medicinal plant, Atomic Absorption Spectroscopy

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1. INTRODUCTION

Wild medicinal plants have provided copious leads to fight diseases, from the beginning of civilization. A considerable proportion of tribal population is still under-nourished and the people living in remote areas cannot produce enough food grains to meet the yearly requirement (Balemie and Kebebew, 2006). *Dillenia Pentagyna* Roxb. (Dilleniaceae) is an herbal plant, commonly known as 'agai' and widely distributed randomly in the Terai belt of North East states of India. Unripe fruits are used as vegetable or sourness and ripe fruits in eaten (Yadav et al., 2015; Gandhi and Mehta, 2013). Fruits are rich in antioxidants that help in lowering incidence of degenerative diseases such as cancer, diabetes, arteriosclerosis, inflammation, brain dysfunction and acceleration of the ageing process (Gordon, 1996; Halliwell, 1996). This plant has been used by tribal and folk communities in various regions. Fruits of *D. pentagyna* are also eaten raw but not very much well known by the people (Dubey et al., 2009; Pradhan and Badola, 2008). Catalase, peroxides, super-oxide dismutase (SOD) and the non-enzymatic antioxidant compounds such as phenols, ascorbate and glutathione is a synergistic antioxidant defensive system, whose combined purpose is to protect cells from active oxygen damage (Nimse and Pal, 2015). Following a harvest period fruits can have a relatively short shelf life during which they undergo profound changes in texture, color and flavor (Glew et al., 2003). The present study showed that biochemical changes affected the nutritional value from unripe to ripening period. Antioxidants terminate the chain reactions by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols. Ascorbic acid (Vitamin C) is the

most important vitamin in this fruit. Vitamin C is required for the prevention of scurvy and maintenance of healthy skin, gums and blood vessels.

Minerals are a respected or valuable chemical substance that is formed naturally in the pulverized. Iron, Zinc, Copper, Ni considered as micronutrients while Mg, Na, K and Ca considered as macronutrients. Iron is important for a wide range of cellular functions (Ibrahim et al., 2018). The proximate analysis determines only the moisture content, total solid content, ash content, total fiber content, total fat content, carbohydrate content, reducing and non-reducing content. This research focused on analysis and comparison of the antioxidant and nutritional properties among four maturation stage, grown in the Terai region area, Bhinga forest region of Shravasti district, U.P., India.

The fruit quality attributes were determined and analyzed as indicators for fruit development, fruit, fresh weight, fruit color and sugar-acid ratio of fruits. The common fruit characteristics are compared with changes in the malondialdehyde and phenolic compounds.

2. MATERIALS AND METHODS

The fruits of Dillenia pentagyna were collected from the Terai region area, Bhinga forest region of Shravasti district, U.P., India.

The fruits were harvested after different maturation stages at 10^{th} day, 25^{th} day, 40^{th} day and 55^{th} day after full bloom. The greening of the fruits increased with increasing days up to 45^{th} days after that fruits were going to the maturity stage.

The color of the fruits was yellowish green at 60^{th} day of harvest. Each parameter was measured in three replications. For enzymatic and non-enzymatic analysis, fruit tissues (200 mg each) were homogenized in 2 ml of 100 mM potassium phosphate buffer, pH 7.5 containing 1 mM of EDTA in the presence of pinch of Poly Vinyl Polypyrrolidone (PVP). All steps in the preparation of enzyme extract were carried out at 0-4ºC.

Proximate analysis

The samples were analyzed for proximate compositions which include moisture content, fat, ash, protein, fiber, flavonoids, carbohydrates and sugar contents. The moisture and dry weight content in the food items were determined by measuring the amount of water removed from the food (AOAC, 1998). It was done by direct heating the food in an Air oven at 100-105^{o}C to constant weight. Ash in the food samples was estimated by heating the dried sample in a Muffle furnace at 600^{o}C for 3h (AOAC, 1998).

Mineral content assay

The collected samples were oven dried at 70-80 ºC for 24h. Dried fruit powder (0.5 g) were digested in H_2SO_4 and $HClO_4$ in 5:2 ratio upto 20 ml, using Digestion System (Kelplus-Classic DX) after that we makeup in 100 ml volumetric flask from distil water (DW). Minerals content was estimated by Atomic Absorption Spectrometer PHILIPS PU 9200X and Flame photometer 128. The results obtained were expressed as mg kg^{-1} of dry weight (dw).

Determination of the total phenolic content

Total phenols were measured according to the Velioglu, Mazza, Gao and Oomah, (1998) using Folin-Ciocalteu reagent. The 200 mg of fruit tissue was extracted with 2 ml of 50% methanol for two hours at extensive temperature. The mixture was centrifuged for 15 min at 15,000 rpm and supernatant was decanted into 5 ml vials. A 500 µl of the extract was mixed with 2 ml Folin-Ciocalteu reagent and allowed to stand for 5 min before the addition of 1 ml of 20% sodium carbonate. After 90 min, absorbance was measured at 750 nm using a UV- spectrophotometer. Total phenols were quantified from a calibration curve obtained by measuring the absorbance of known concentrations of Gallic acid.

Determination of lipid peroxidation content

The level of lipid peroxidation in plant tissues was determined as 2-thiobarbituric acid reactive (TBARs) metabolites mainly

malondialdehyde (MDA). The MDA is one of the most final decomposition products of lipid peroxidation and has been used as an index for lipid peroxidation status (Heath and Packer, 1968). Briefly, 0.5 g of sample was homogenized in 1mL 0.5% trichloroacetic acid (TCA). The homogenate was centrifuged at 19,000 g for 20 min. The 0.5 ml supernatant was mixed with 2.5 ml 20% TCA containing 0.5 % TBA, heated in boiling water bath for 30 min and then allowed to cool rapidly in a nice bath. The supernatant was centrifuged at 10,000 rpm for 10 min and the resulting supernatant was used for determination of MDA. The concentration of MDA was calculated from the absorbance at 532 nm by using the extinction coefficient of 155 m/M cm.

Determination of Antioxidant activities

0.5 g of fruits was homogenized in 1.5 ml of respective extraction buffer in a pre-chilled mortar and pestle. The homogenate was centrifuged at 15,000 rpm for 10 min at 4 ºC before determination of antioxidant enzyme activities. SOD activity was assayed by monitoring the inhibition of photochemical reduction of nitroblue tetrazolium (NBT) according to the method of Beyer and Fridovich (1987). Fruits were homogenized in 1mL cold 100 mM K-phosphate buffer (pH-7.8) containing 0.1 mM ethylene diaminetetraacetic acid (EDTA), 1% (w/v) polyvinyl-pyrrolidone and 0.5% (v/v) Triton X-100. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the reduction of NBT as monitored at 560 nm. For the determination of APX, fruits were homogenized in 100mM Na-phosphate buffer (pH 7.0) containing 5 mM AsA, 10% glycerol and 1 mM EDTA. APX activity was determined in 1 ml reaction mixture containing 50 mM K-phosphate buffer (pH-7.0) and 0.3 mM H_2O_2. The decrease in absorbance was recorded at 290 nm for 3 min (Chen and Asada, 1989). For GR, fruits were homogenized in 100 mM sodium phosphate buffer (pH-7.0) containing 1 mM EDTA. GR activity was assayed by following the reduction of 5, 5'-dithio-bis 2-nitrobenzoic acid (DTNB) at 412 nm (extinction coefficient, 13.6 mM/cm) with some modifications as described by Smith, Vierheller and Thorne (1988). The assay mixture (1 ml) contained 100 mM K-phosphate buffer (pH-7.5), 1 mM oxidized glutathione (GSSG), 1 mM EDTA, 0.08 mM DTNB, 0.1 mM NADPH and 100 ml of enzyme extract. POD activity was measured by following the change of absorption at 470 nm due to guiacol oxidation (extinction coefficient, 26.6 mM/cm).

Estimation of Amino acid by Gas Chromatography

The extraction of amino acids (AA) content from plant samples were followed by the method of Weckwerth et al. (2004). To extract AA, 200 mg of plant tissues was ground using liquid nitrogen until the fine powder with the help of the chilled mortar and pestle. The ground tissues were dissolved in 1.5 ml of cold solvent containing methanol: chloroform: water in 5:2:1 ratio (stored at -20°C).

The homogenized plant tissues were kept on ice for 30 min with alternate shaking using orbital shaker. The homogenate was centrifuged at 12,000 rpm for 10 min and the supernatant was collected carefully. The collected samples were redissolved in deionized water and chloroform. The solution was vortex-mixed and upper phase was collected carefully and dried in a vacuum oven. The vacuum dried samples were used for the derivatization process.

The Amino acid was derivatized and measured by the method of Sobolevsky et al., (2003). The samples were heated at 70°C for 30 min and 1µl of derivatized sample was subjected to the gas chromatography. Samples were analyzed by the gas chromatography (Agilent GC model 7890A) with FID using capillary BP-5 column (5% phenol methyl polysiloxane column, 30 m x 0.32 mm x 0.25 µm). The injector and detector temperatures were maintained at 280°C. The initial oven temperature was kept 70°C for 2 min and increased to the final temperature of 300°C with 5°C min^{-1}.

The relative percentage amount of each component was calculated by comparing its average peak area to the total areas.

Statistical analysis

Statistical analysis was carried out under a completely randomized design with three independent experiments. All the experiments were carried out in three replicate ± S.D. The data were analyzed by two way analysis of variance (ANOVA) to confirm the variability and validity of results, and Duncan's multiple range test (DMRT) was performed to determine significant differences between treatments.

3. RESULTS AND DISCUSSION

Changes in proximate content

At the four maturation stage, fruit weight and moisture content were gradually increased during maturation reaching the maximum level on the 55th day. The moisture, ash, reducing and non-reducing sugar content increased with increasing days of the fruit maturity with minimum in 10th day and maximum in 55th day. Ash and sugar content positively correlated with increasing the humidity. While on the 55th day, protein, carbohydrate, crude fat and total solid content sharply decreased with the increasing days of maturity (Table 1).

Sugar is one of the biochemical components of fruit virtue and their kinds and amount directly impression fruit-palate components such as mellowness (Moriguchi et al., 1992). The results are corroborated by the findings of Upadhyay et al. (2001). Reducing sugar and non-reducing sugar content was augmented during the maturation period. The high carbohydrate content was recorded highest 0.91 g/100 g dw on day 25th d and lowest 0.35 g/100 g dw on 55th day. Protein content was found highest 0.92 g/100 g fw on the 10th day and lowest 0.24 g/100 g fw on 55th day.

Changes in mineral content

Minerals are dietary requirements for humans and exert various physiological effects. Potassium (K) and Calcium (Ca) are minerals that are essential for controlling the salt balance, bone structure, and functions of the human body. Mg is also useful to the body as a minor component of bones and plays a catalytic role in respiration (Yim et al., 2015). Individuals who suffer from a nickel allergy should avoid foods rich in this element. For micro-minerals, Zn is especially important for the normal functioning of the immune system, and Fe is the major component of essential biological compounds such as transferrin, ferritin, and haemoglobin (Brody, 1994). The mineral analysis was carried out from this fruit were Sodium (35.90 mg/100g to 48.60 mg/100g), Potassium (41.45 mg/100g to 31.10 mg/100g), Calcium (23.47 mg/100g to 39.86 mg/100g), Magnesium (68.51 mg/100g to 53.98 mg/100g) and micronutrients i.e. Copper (5.25 mg/100g to 5.82 mg/100g), Manganese (4.28 mg/100g to 3.76 mg/100g), Zinc (0.92 mg/100g to 2.64 mg/100g), Iron (9.40 mg/100g to 4.60 mg/100g), and Nickel (2.26 mg/100g to 2.82 mg/100g) (Table 2).

Table 1: Description of the proximate content value of *Dillenia pentagyna* fruits derived from selected maturation stages. Each value in the table was obtained by calculating the average of three determinants (n=3) and data are presented as Mean ± SD

Proximate parameters	10 days	25 days	40 days	55 days
Moisture content (%)	82.41±0.93	82.69±0.87	87.65±0.99	87.99±0.81
Total solid content (%)	17.58±0.75	17.30±0.85	12.35±0.89	12.01±0.48
Ash content (%)	6.57±0.67	6.98±0.38	7.23±0.43	8.56±0.57
Crude fat content (%)	5.98±0.51	5.72±0.42	4.59±0.83	4.14±0.59
Protein content (g/100 g Fw)	0.92±0.06	0.56±0.07	0.43±0.02	0.24±0.01
Flavonoid content (g/100 g dw)	1.62±0.08	0.79±0.07	1.18±0.05	0.53±0.01
Carbohydrate content (g/100 g dw)	0.73±0.06	0.91±0.07	0.46±0.05	0.35±0.04
Reducing sugar content (g/100 g dw)	0.80±0.04	0.93±0.02	1.16±0.02	1.59±0.07
Non-reducing sugar content (g/100 g dw)	1.18±0.13	2.17±0.17	2.75±.09	2.95±0.12

Table 2: The changes in mineral content during four maturation stage of *D. pentagyna* fruits. All values are introduced as mg/100g dw

Nutrients		10 days (mg/100g)	25 days (mg/100g)	40 days (mg/100g)	55 days (mg/100g)
Macro Nutrients	Sodium (Na)	35.90	38.01	41.20	48.60
	Potassium (K)	41.45	40.65	32.60	31.10
	Calcium (Ca)	23.47	28.31	35.10	39.86
	Magnesium (Mg)	68.51	61.28	56.42	53.98
Micro Nutrients	Copper (Cu)	5.25	5.34	5.40	5.82
	Manganese (Mn)	4.28	4.28	4.26	3.76
	Zinc (Zn)	0.92	1.92	1.24	2.64
	Iron (Fe)	9.40	5.61	5.20	4.60
	Nickel (Ni)	2.26	2.23	2.64	2.82

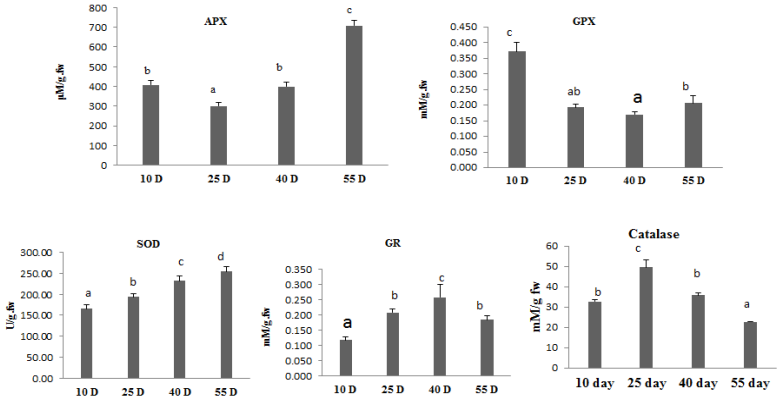

Fig. 1: Changes of the antioxidant enzyme contents in *Dillenia pentagyna* fruits derived from selected maturation stages

Changes in enzymatic antioxidant activities

The results of ascorbate peroxidase (APX), peroxidase (POD), and superoxide dismutase (SOD) and glutathione reductase (GR) content of different maturation stage of *D. pentagyna* fruits were significant and shown in Fig. 1.

All values are mean of four replicates ±SD and values marked with similar symbols are non-significantly different (Duncan's test, p≤0. 05).

The fresh fruit extract content range was APX (353.33 µM/g Fw-708.66 µM/g fw), POD (0.257 µM/g fw-0.207 µM/g fw) and GR (0.139 µM/g fw-0.191 µM/g fw) while SOD ranged from 192.53 to 253.92 U/g fw. Enzymatic antioxidant compounds and phytochemicals are found in plants that are not required for normal functioning, but have a beneficial effect on health or play an active role in the amelioration of diseases.

In fact, some people claim that many of the diseases afflicting human beings are the result of lack of phytonutrients in their diet Ayoola et al., 2008. During ripening, a Phyto-hormone ethylene is released which activates the transcription genes for the synthesis of various enzymes which degrade the Phyto-constituents and involve in the ripening process. The metabolism will work in the proper way and also a number of free radicals generated.

Changes in non-enzymatic antioxidant activities

The total phenol content in the fruit extracts in all four stages expressed as Gallic acid equivalent (GAE) from 0.352 to 0.702 mg GAE/g dw with the lowest at 55th day and highest at 25th day.

Among the various natural antioxidants, phenolics are very important constituents because of their multiple biological effects and direct contribution to the antioxidative activity (Lee et al., 2002).

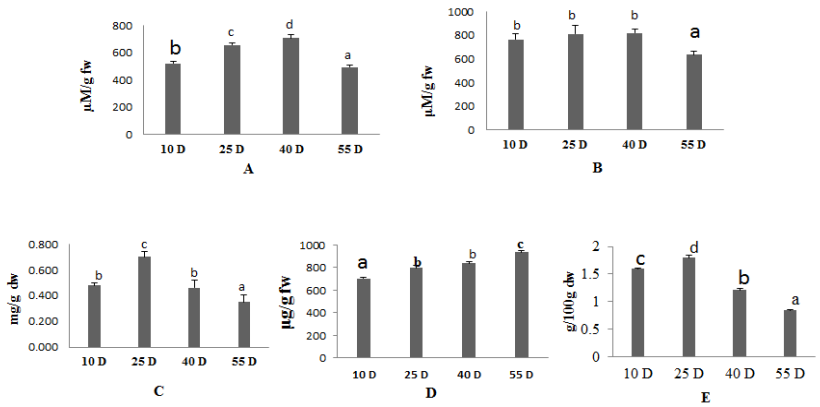

Fig. 2: Changes of the non-enzymatic antioxidant such as A. Ascorbic acid B. Glutathione C. Total phenolics D. Tocopherol E. Flavonoids in *D. pentagyna* fruits at selected maturation stages

The results revealed that there is a strong coincidence between antioxidant activity and phenolic content. Several studies on total phenolic contents were published over the years demonstrating its importance in the medicinal field (Abdalbasit et al., 2009). The gradual decrease in Vitamin C content was recorded with increasing maturity stage of the fruits sowing the maximum value of 946.42 µmol/g fw at 10th day and minimum 487.13µmol/g fw at 55th day (Fig. 2).

All values are mean of four replicates ±SD and values marked with similar letters are non-significantly different (Duncan's test, p≤0.05).

A similar trend was also recorded in lipid content with highest amount 24.98 µmol/g fw at 10th d and lowest as 11.54 µmol/g fw at 55th d. Natural antioxidants, which are ubiquitous in fruits, vegetables and medicinal plants have received great attention and have been studied extensively, since they are effective free radical scavengers (Ratnam et al., 2006).

Changes in amino acid concentration of maturation in Dillenia pentagyna fruits

The amino acids analyzed from the fresh tissue extract of *D. pentagyna* fruits using Gas chromatography. The analysis was done using Agilent (GC model 7890A) with FID using capillary BP-5 column (5% phenol methyl polysiloxane column, 30 m x 0.32 mm x 0.25 µm). The concentration of L-Alanine increased from 1.157% to 7.624%, Glycine, from 3.019 to 8.157, Leucine, from 2.022% to 4.524%, Proline, from 2.209 to 2.842, Methionine, from 1.843% - 4.393%, L-Asparagine, from 1.738% to 6.841% in fresh fruits increased while L-Valine, from 7.499 % to 1.632%, Isoleucine, from 4.490% to 2.910%, Aspartic acid, from 3.281% to 1.483%, L-lysine, from 6.875% to 1.086%, L-tyrosine, from 3.633% to 2.107% decreased with increasing date of maturity from 10 to 55 days. However, Glutamine (2.181% - 2.817%) and Phenylalanine (1.012% - 2.540%) did not show any trend between maturity stage (Table 3).

4. CONCLUSIONS

The present study shows that are good source of many bioactive compounds which possess various economic effects on human health. Health promoting total phenolic and peroxide compounds, other than malondialdehyde *e.g.* flavonoids, super oxides has contributed positively to the total antioxidant activity and thus might be implemented in further breeding study programs. As a result, determining the biological properties of plants used in traditional medicine is helpful to the rural communities and informal resolutions. Important information has been generated on antioxidants and nutritional properties of the samples of *D. pentagyna* as they change with maturation time and system. It can be concluded that these qualities generally change with time.

Table 3: The changes in amino acid concentration value during four maturation stages of *D. pentagyna* fruit extracts using GC

S.No.	Rt	Amino Acids	Formula	10th days % Area	25th days % Area	40th days % Area	55th days % Area
1	6.85	L-Alanine-2TMS	$C_9H_{23}NO_2Si_2$	1.157	5.519	6.354	6.624
2	12.32	Glycine-2TMS	$C_8H_{21}NO_2Si_2$	3.019	6.817	7.157	3.102
3	9.90	L-Valine-TMS	$C_8H_{19}NO_2Si$	7.499	7.591	3.711	1.632
4	10.92	Leucine-2TMS	$C_{12}H_{29}NO_2Si_2$	2.022	3.436	3.765	4.524
5	11.53	Isoleucine-2TMS	$C_{12}H_{29}NO_2Si_2$	4.490	3.459	2.910	3.299
6	13.39	Proline	$C_{11}H_{23}NO_3Si_2$	2.209	2.507	1.661	2.842
7	18.51	Aspartic acid-3TMS	$C_{13}H_{31}NO_4Si_3$	3.281	2.103	1.850	1.483
8	19.50	Methionine-2TMS	$C_{11}H_{27}NO_2Si_2$	1.843	2.224	2.837	4.393
9	21.16	Glutamine-3TMS	$C_{14}H_{33}NO_4Si_3$	2.698	2.181	2.682	2.817
10	22.18	Phenylalanine-2TMS	$C_{15}H_{27}NO_2Si_2$	1.862	2.540	1.0124	1.482
11	23.34	L-Asparagine-2TMS	$C_{10}H_{24}N_2O_3Si_2$	1.738	4.787	6.039	6.841
12	26.62	L-lysine	$C_6H_{14}N_2O_2Si_2$	6.875	5.459	3.645	1.086
13	29.71	L-tyrosine	$C_9H_{11}NO_3Si_2$	3.633	2.365	2.159	2.107

Thus, we may conclude that peels of fruits are an effective resource of antioxidants and amino acids, which can be effectively utilized in food and pharmaceutical industries.

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