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NUTRITIVE AND NUTRACEUTICAL CHARACTERISTICS IN FRUIT MESOCARP OF PALMYRA PALM *(BORASSUS FLABELLIFER* L.)

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The present investigation was mainly focused on the evaluation of the nutraceutical potential of the fruit pulp of Palmyra palm *(Borassus flabellifer* L.) with biochemical and analytical methods. Proximal composition analysis of palmyra fruit pulp (PFP) at different stages of fruit development and ripening revealed considerable increase in the amount of major nutrients such as proteins, total sugar (reducing and non- reducing) and total carbohydrate. Crude fibre was found in appreciable amount (68mg /g), while the ash content was very low (6 mg / g tissue). Besides essential nutrients, PFP contained some secondary metabolites of least nutritive value (antinutritional factors) such as tannin ranged from 13.39 mg/g to 12.56mg/g, phytic acid 1.96mg/g to 8.95 mg/g, oxalate (4.95 to 8.34%), saponin 16.3 to 8.3% and polyphenols 288mg/g to 48mg/g at 2WAP and 14 WAP, respectively. Interestingly the level of tannin, saponin and total phenols decreased gradually while the value of phytate and oxalate increased considerably. RP-HPLC chromatogram of PFP phenolics showed the presence of valuable phenolic acids such as cinnamic, coumaric, chlorogenic, ferulic, gallic, hydroxy benzoic, caffeic and para catechol. Subsequently, PFP was subjected to chemical analysis for the isolation of natural antioxidants. Remarkable levels of pro vitamin A (β-carotene) 62.3µg/g, vitamin C (ascorbate) 2640 µg/g and vitamin E (tocopherol) 58.3 µg/g were obtained .A progressive increase in all antioxidants was noticed at various stages of fruit development. Higher level of vitamin A, C and E together with phenols, phenolic acids, tannins and phytate make the fruit a potential nutraceutical food. The total antioxidant activity of PFP was further checked by FRAP assay and showed a high value (2683µ mol / L). The fruit pulp was also a good source of nitrogen, phosphorus, calcium, magnesium, potassium, sulphur, iron, manganese, zinc and copper. The last three elements function additionally as antioxidants and protect specific regions of enzymes from the radical attack and thus preserving its stability and activity.

Keywords : Amino acids; Antioxidants; Antinutritional factors; Ascorbate; β-carotene; Nutritional; Nutraceutical; Oxalate; Palmyra fruit pulp (PFP); Polyphenols; Phenolic acids; Phytic acid; Saponin; Tannins; Tocopherol.

Introduction

Biomedical and Epidemiological research strongly suggested that diets play an important role in the prevention of chronic diseases such as cardiovascular diseases, cancer, diabetes, and Alzheimer's disease[1-3]. Regular consumption of fruits and vegetables has been associated with reduced risk of chronic diseases[4-7]. This may be due to the occurrence of some phytochemicals that combat oxidative stress in the body by helping to maintain a balance between oxidants and antioxidants. Recent research has shown that mixture of phytochemicals in foods provides better protective health benefits than single phytochemicals through a combination of additive and/or synergistic effects[8]. About 5000 of phytochemicals have been identified in plants, and still a large number remains unknown[9]. The structure and compositions of phytochemicals may vary with plants and thus offer different protective mechanisms. Hence, for the maximum health benefits, sufficient amounts of phytochemicals from fruits, vegetables, and whole grain-based foods are recommended.

Palmyra *(Borassus flabellifer* L.), also known as the jaggery palm or toddy palm is a tropical palm; flourish luxuriantly in the dry lands of many tropical countries. In India this multipurpose tree of great utility grows extensively in Tamil Nadu, Andhra Pradesh, Orissa, West

Bengal, Bihar, Karnataka, Kerala and Maharashtra. Palmyra is a dioecious palm which yields an array of economic products such as immature endosperm, mesocarp pulp, tuberous seedlings, and sweet sap from the inflorescence, toddy, palm sugar, brush fibre and wood. Palmyra fruit pulp (PFP), a sweet or little bitter edible portion, which is available in abundance, is largely unutilized owing to the presence of a bitter principle[10]. The bitter principle was identified as a steroidal saponin tetraglycoside flabelliferin-II with MW 1030 D[11].The sweet pulp of some varieties can be used for making jams and cordials. Research on PFP has been largely confined to the isolation and purification of different groups of flabelliferins[12, 13]. No serious attempt has been carried out elsewhere to analyze the nutritional, antinutritional and antioxidant characteristics in PFP. Hence, the present study aims to analyze the fruit as a whole, biochemically to reveal the nutraceutical potential.

Material and Methods

Fruits were collected from different locations of Thiruvananthapuram (Kerala) and Kanyakumari (TN) districts. The stage of growth of the fruit was calculated as weeks after pollination (WAP).All biochemicals were obtained from Sigma Chemical Co., St. Louis, MO, USA and other chemicals were of analytical grade. The fruits were analyzed for proximate composition using standard methodologies. Crude protein was obtained by multiplying the total nitrogen content by a factor value proposed by Pearson[14].Carbohydrates and reducing sugar were determined by the method of Mohan and Janardhanan[15]. Mineral contents were analyzed from the fruit tissue by atomic spectroscopy[16]. Quantitative analysis of tannins was carried out spectrophotometrically using Folin- Dennis reagent. Extraction was done with methanol. Total phenol contents of fruit tissues were estimated by the method of Mayr *et al.*[17]. Phenolic components of extracts were separated using HPLC following the method of Beta *et al.*[18]. HPLC system (Waters Associates) equipped with a 7725 Rheodyne injector and Waters 510 HPLC pump, 486 tunable absorbance detector and Millennium 2010software data module were used for the study. HPLC column of 4.6×250 mm, reverse phase (RP) C8 was used for the fractionation of phenolic acids. Potassium hydrogen phosphate and acetonitrile in the ratio of 75:25 was used as the mobile phase for the isocratic elution. An elution period of 20 min with a flow rate of 0.8ml/min was given. Sample (10 µl) was injected and the absorbance at 254 nm was recorded. Standard phenolic acids such as gallic, vanillic, p-hydroxybenzoic, ferulic, chlorogenic, sinapic, paracoumarate and cinnamic acids were injected into the column separately. Comparing with the retention time of the standard phenolic acids, various phenolic acids in the sample were identified. Heights of peaks were taken for quantification.

Ascorbic acid was quantitatively determined according to 2, 6-dichlorophenolindophenol dye method[19]. The ascorbic acid of fresh samples (10g) was extracted by grinding in a suitable medium with a small amount of sand and using 3% meta phosphoric acid (v/v) as a protective agent. The extract was made up to a volume of 100ml mixed and centrifuged at 3000g for 15 min at room temperature. Ten milliliters was titrated against standard 2, 6-dichlorophenolindophenol dye, which was already standardized against standard ascorbic acid. Results were expressed as $mg100g^{-1}$ on fresh weight (fw) basis. The β-carotene content of fruit samples were estimated by Soxhlet method using the solvent hexane[20]. 20 ml of 60% potassium hydroxide was added to the sample (1 gm) sample taken in a conical flask and kept in darkness for 3 hrs. The mixture was transferred to a separating funnel and the ether layer was collected after thorough washing with petroleum ether (6 times) and the absorbance was recorded at 429 nm using petroleum ether as blank.

The tocopherol content of the fruit samples was estimated by the method of Paquot and Hautfenne[21]. 1g tissues was refluxed in ascorbic acid and ethanol mixture for 3 to 5 min. 1ml of potassium hydroxide and water mixture was added and boiled for 3 min. The mixture was cooled and 25 ml distilled water was added and transferred to a separating funnel. 50 ml of diethyl ether was added and shaken for 1 min. The lower aqueous layer was collected and washed three times with ether. The tocopherol content was dissolved in ether and the ether layer was washed with distilled water until it becomes alkali free. Ether layer was removed finally by evaporation. The residue was redissolved in 5ml of benzene ethanol mixture and the extract was evaporated. The dry extract was then dissolved in 1ml of hexane and was again evaporated. The residue was finally dissolved in 4ml ethanol. The absorbance was measured at 292 nm and the tocopherol was determined using the standard curve of tocopherol.

Antioxidant activity was estimated as per assay method of Benzie and Strain[22]. Ethanolic extract (100µl) were added to 3 ml of ferric reducing ability of plasma (FRAP) reagent [10mM 2,4,6-tripyridyl S-triazine (TPTZ) in40 mM HCl and 20 mM ferric chloridein 300 mM sodium acetate buffer, pH3.6 in the ratio of 1:1:10 (v/v)] and mixed thoroughly and absorbance noted after 4 min at 593 nm against water blank. Calibration was against a

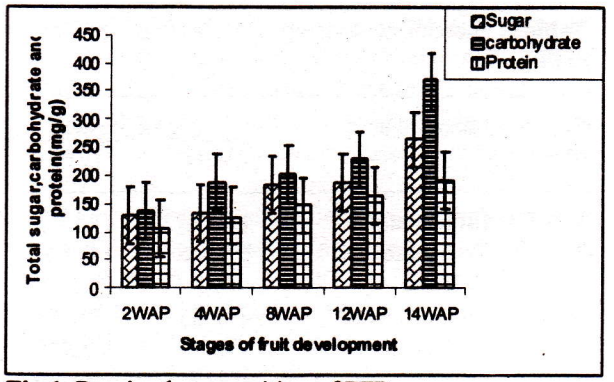
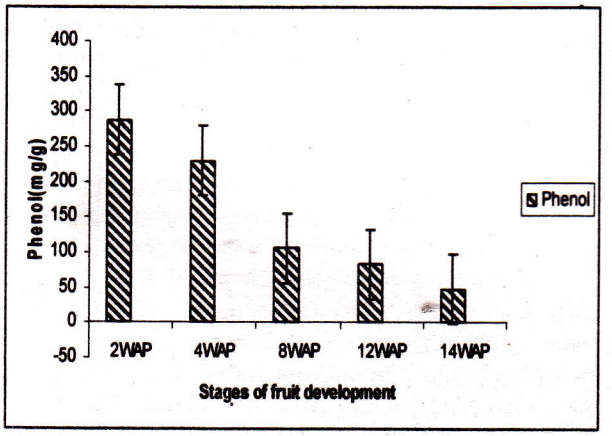


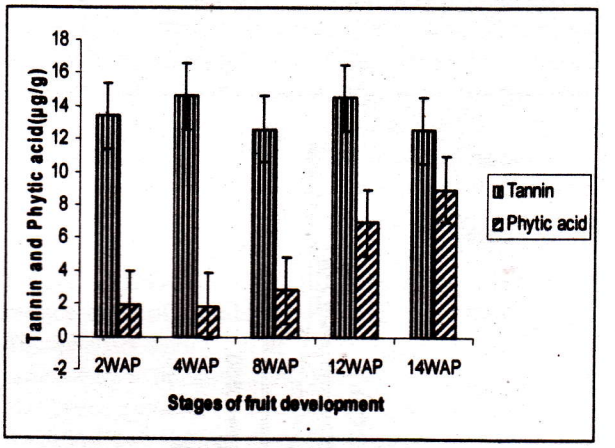
Fig.1. Proximal composition of PFP.

Table 1. Aminoacid profile of Palmyra Fruit Pulp (PFP)

Free amino acids	(mg / g tissue)
Tyrosine	1.429
Phenylalanine	0.1963
Serine	0.2286
Glycine	0.1777
Aspartic acid	0.6870
Proline	0.9732
Cysteine	0.872
Isoleucine	0.1184



(a) Total Phenol



(b). Tannin and Phytic acid

Fig.2. (a &b). Antinutritional factors in Palmyra fruit pulp (PFP).

standard curve 950-100µM ferrous ion produced by the addition of freshly prepared ammonium ferrous sulphate. Values were obtained from three replications and expressed as µmol FRAP/g fresh weight.

All determinations were done in replicates of 10 except for mineral analysis (triplicate). Pearson's coefficient and regression analysis were carried out to correlate the total phenol content and antioxidant activity.

Results and Discussion

Proximal composition: The results of the proximal composition analysis of PFP at different stages of fruit development and ripening revealed considerable increase in the amount of major nutrients such as proteins, total sugar (reducing and non- reducing) and total carbohydrate (Fig.1). The level of protein ranged from 106 mg/g to 192.48mg / g tissue FW at 2 weeks after pollination (WAP) and 14WAP, respectively. The amount of protein was substantiated by the profile of major amino acids (Table 1). It is observed that tyrosine, proline and cystine are the most abundant amino acids and others in substantial amounts. The total sugar value varied from 129mg / g to 264 mg / g tissue FW. The value of sugar was found to be increasing considerably and reaching the peak at 14WAP *i.e.,* in the last stage of fruit development. A progressive increase in the level of total carbohydrate (138 mg/g to 368mg/g) was also observed at various stages of fruit maturation. In fully ripe fruit, crude fibre was found in appreciable amount (68mg /g), while the ash content was very low (6mg/g tissue). The higher amount of the essential nutrients in PFP makes it as a valuable fruit.

Mineral composition : PFP is a rich source of major mineral nutrients such as N 1.01%, P 0.125%, K3.8%, Ca 0.6%, Mg 0.28%, and S 0.07% (Table 2). The minerals such as Fe, Mn, Cu and Zn were also present in moderate quantity. Among them, Zn (12.5 ppm) showed lowest value and Fe (151ppm) highest value. Cu, Zn and Mn are essential components of numerous enzymes that catalyze oxidative- reduction reactions and is also required for collagen synthesis and iron mobilization[23]. The divalent cations Mg^{++}, Mn^{++} are cofactors for many enzymes[24]. Zn,

Table 2. Mineral nutrients in PFP.

Nutrients	Quantity
N	1.01 %
P	0.12 %
K	3.80 %
Ca	0.60 %
Mg	0.28 %
S	0.07 %
Fe	151 ppm
Mn	43 ppm
Zn	12.5 ppm
Cu	14.4 ppm

Table 3. Phenolic acids composition of Palmyra fruit pulp (PFP).

Sl. No.	Phenolic acids	(µg /g tissue) 2WAP	14WAP
1	Cinnamate	197.83	5.67
2	Coumarate	678.96	451.15
3	Caffeate	ND	4.34
4	Chlorogenate	471.63	77.63
5	Ferrulate	135.79	90.23
6	Gallate	377.3	62.10
7	Hydroxy benzoate	10.99	0.315
8	Paracatechol	5.35	ND
9	Vanillate	ND	ND

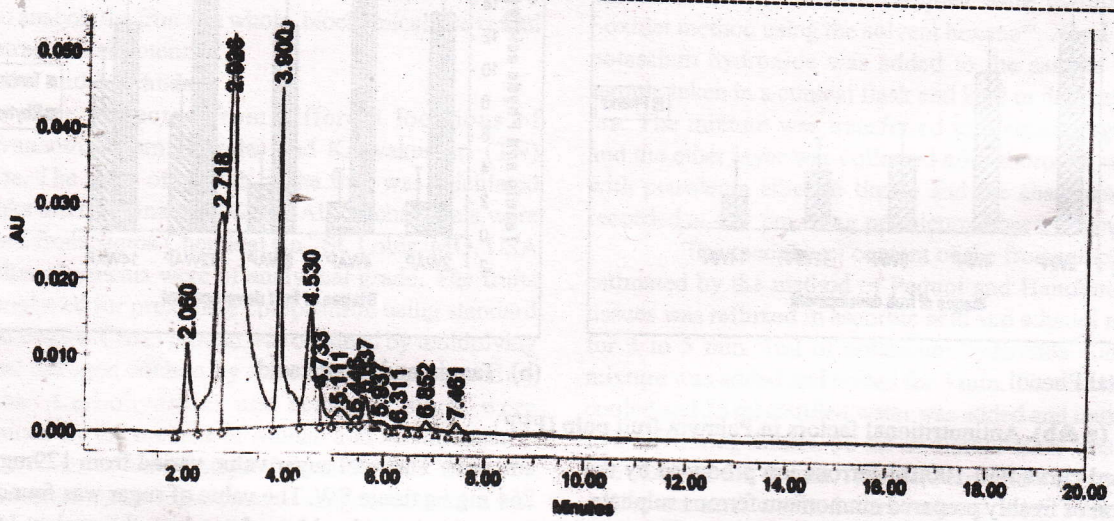
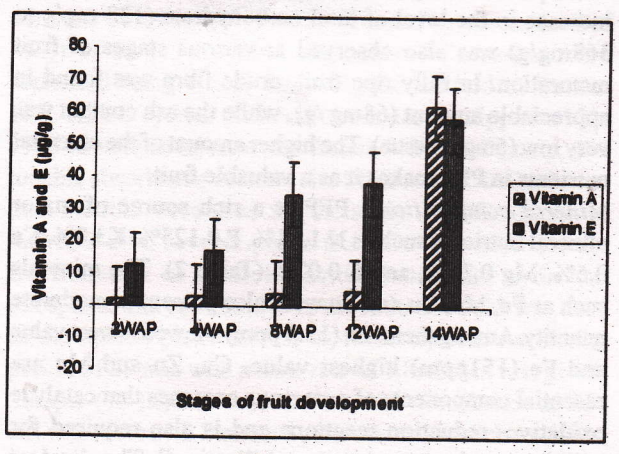
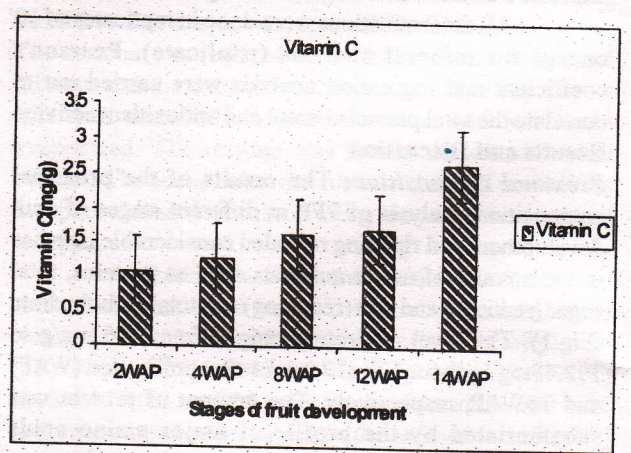


Fig.3. RP-HPLC chromatogram of PFP showing the profile of phenolic acids.



(a)



(b)

Fig.4 (a & b). Antioxidant characteristics (Vit A, E & C) in PFP .

Cu and Mn act as protective against oxidative stress by scavenging superoxide anion to H_2O_2 and thus averting cellular damages[25]. Thus, these mineral elements indirectly and additionally function as antioxidant. Therefore, the wild species can be ideal source of the mineral supplement in the daily diet.

Antinutritional constituents: Besides essential nutrients, PFP contained some secondary metabolites of least nutritive value (antinutritional factors) such as oxalate (4.95 and 8.34%), saponin (16.3 and 8.3%), tannin (13.39mg/g and 12.56mg/g), phytate (1.96mg/g and 8.95 mg/g) and polyphenols (288mg/g and 48mg/g) at 2WAP and 14 WAP, respectively. Interestingly the levels of saponin, tannin and total phenols decreased gradually while the value of oxalate and phytate increased considerably (Fig.2). Oxalate can bind calcium present in food thereby rendering calcium unavailable for normal physiological and biochemical role such as maintenance of strong bone, teeth cofactor in enzymic reaction, nerve impulse transmission and as clotting factor in blood[26]. The calcium oxalate, which is insoluble, may also precipitate around the soft tissues of kidney causing kidney stones[27]. High level of saponin has been associated with gastroenteritis manifested by diarrhea and dysentery.Tannin is an antinutrient that binds and precipitates proteins. Recently tannins have also been studied for their potential effects against cancer through different mechanisms[28]. Phytin is a complex salt of calcium and magnesium, and it may reduce the bioavailability of minerals such as calcium, magnesium, zinc and iron. Phytic acid and its mineral binding properties may also prevent colon cancer by reducing oxidative stress in the lumen of the intestinal tract.Tannin, phytin and cyanide significantly influence the function and nutritional properties of foods[29]. Phenolic compounds are a largest group of phytochemicals that have shown disease preventing and health promoting effect and have received major attention among researchers largely due to their antioxidant activity. This wild species was found to be potential source of polyphenolics. The gradual reduction in the level of total polyphenols may be linked to the active lignin biosynthesis and defense mechanism during the early stages of fruit development. However, from the nutritional point of view, the main concern with phenols, in dietary proteins, is the way in which they decrease its digestibility and nutritive value by decreasing the activity of digestive enzymes such as amylase, trypsin, lipase and also reduce the absorption of vitamin B_{12}[30]. RP-HPLC chromatogram of PFP phenolics showed the presence of valuable phenolic acids such as cinnamic, coumaric, chlorogenic, ferulic, gallic, hydroxyl benzoic, caffeic acid and para catechol with potent antioxidant property (Fig.3). A significant variation was observed between the young (2WAP) and mature (14WAP) fruits with regard to the value and type of phenolic acids (Table 3). In nutritional point of view, the higher content of both total phenolics and tannin are not desirable to human consumption. But recently, the phenolic constituents of various plants have shown potential medicinal properties, including antioxidant activities[31, 32].

Antioxidants: Subsequently, PFP was subjected to chemical analysis for the isolation of natural antioxidants. Fig.4. displays the antioxidant vitamins pro vitamin A (β-carotene), vitamin C (ascorbate) and vitamin E (tocopherol). In the present study, remarkable levels of pro vitamin A (β-carotene) 62.3µg/g, vitamin C (ascorbate) 2640 µg/g and vitamin E (tocopherol) 58.3 µg/g were obtained. A progressive increase in all antioxidants was noticed at various stages of fruit development. Carotenes have a positive effect on the immunological system and protect the skin from ultraviolet radiation[33]. In addition to the pro -vitamin A activity, β-carotene has been found to reduce risks of certain cancers especially lung cancer. Tocopherols mainly act as a free radical chain breaking antioxidant in liposomes and cellular membranes, but they also possess reactivity as singlet oxygen quenchers and in repairing free radical damages in proteins. Tocopherol plays a significant role as an antioxidant to protect polyunsaturated fatty acids (PUFAs) and other components of cell membrane and low-density lipoprotein (LDL) from oxidation, therefore preventing heart diseases[34]. Ascorbic acid is perhaps the most important antioxidant in extra cellular fluids *in vitro,* ascorbic acid behaves as an efficient antioxidant in several different ways, for instance by scavenging radicals produced by certain drugs, protecting lung fluids from the damages due to particularly dangerous air pollutants such as O_3 and NO_2^-, reducing lipid peroxidation in cigarette smoke, and scavenging peroxyl, sulphenyl, urate, nitroxide and other radicals[35]. Ascorbate was found to be most effective in inhibiting lipid peroxidation by peroxyl radical initiator and also an effective free radical scavenger involved in regeneration and recycling of vitamin E[36, 37]. Many studies have shown that an adequate intake of vitamin C is effective in lowering the risk of developing cancers and cardio vascular diseases[37]. Higher level of vitamin A, C and E together with phenols, phenolic acids, tannins and phytic acid make the fruit as a potential nutraceutical food. The *in vitro* antioxidant activity (AOX) of PFP was further checked by FRAP assay and exhibited a very high value (2683µ

mol / L) which may be due to the additive action of the various phytochemicals with radical scavenging property. The value is higher than those reported in cultivars of pepper and cabbage[38].

The present investigation, strongly suggest that the palmyra fruit pulp (PFP) is a rich source of essential nutrients especially sugar, protein and fibre content and several secondary metabolites with antioxidant properties including natural antioxidant vitamins. Regular consumption of this cheaply available but valuable food can reduce the risk of many chronic diseases in the village as well as urban communities in the near future.

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