



HYDROGEN CYANIDE AND OTHER ANTI-NUTRITIONAL CONTENTS OF SOME EDIBLE PLANT PARTS COMMONLY AVAILABLE IN MANIPUR

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ABSTRACT

Some of the commonly available fruits, plant parts contain anti-nutritional constituents, which are toxic to the consumers. To validate this information an experiment is planned for the estimation of hydrogen cyanide and other anti-nutritional matters like saponine, phytic acid, cyanide, total and bound phenol, ortho-dihydric phenol, tannin and phytate), from 9 edible plants which are commonly available in Manipur are selected viz., 6 plants from Rosaceae family [*Docynia indica* (Wall.) Decne., *Prunus armeniaca* L., *Prunus domestica* L. var. *Kalen heikha*, *Prunus domestica* L. Var. *Mao Heikha*, *Prunus persica* (L.) Stokes var. light pink flower, *Prunus persica* (L.) Stokes var. deep pink flower], 2 plants from Euphorbiaceae [*Manihot esculenta* var. red cortex and *Manihot esculenta* var. whitecortex] and 1 plant from Leguminosae family [*Phaseolus lunatus*]. Evaluation of nutritive and anti-nutritional value of these selected samples provides an easily accessible natural source that could be used as food supplement. Though all the analysed fruits containsaponins, phytates and tannins, it is found that the edible pulp of the six fruits namely *Docynia indica*, *Prunus armeniaca*, *P. domestica*, *P. persica* are useful for consumption and the seed of these six fruits were dangerous to health if taken at a large quantity. Fresh consumption of *Manihot esculenta* root in large quantity is dangerous to human being.

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INTRODUCTION

Hydrogen Cyanide (Prussic acid) is produced when cyanogenic glycosides in plant cell get rupture and hydrolyzed with enzymes (Franks *et al.*, 2005). The release of hydrogen cyanide (HCN) by plants was firstly ascribed to a particular compound by Robiquet and Charlard who isolated amygdalin from bitter almonds (Vetter, 2000).

All plants contain certain types of flavones and majority of them are used in therapeutic purposes both in Ayurveda and Allopathy medicine (Mayer, 1987). They act as antioxidants, enzyme inhibitors and protection of plant tissues from damaging UV radiation (Croteau *et al.*, 2000). Addition of ascorbic acid, a strong antioxidant and ethylenediaminetetraacetic acid, a metal chelator, totally prevent the loss of phenolic acids during alkaline hydrolysis (Nardini *et al.*, 2002). About 4-57% of the phenolic compounds present in fruits existed in their bound forms and bound phenols are covalently conjugated to cellulose, pectin and polysaccharides through ester bonds (Montilla *et al.*, 2010).

The phosphorus in phytic acid is not nutritionally available to the monogastric animals. Phytic acid also interferes with calcium and iron absorption (Thimmaiah, 1999).

MATERIALS AND METHODS

Source of Plant Materials

Four plants of *Rosaceae* family with two varieties of *Prunus domestica* L. and two varieties of *Prunus persica* (L.) Stokes, a species of *Euphorbiaceae* family with two varieties and a species of *Leguminosae* family were selected for the present study. Four species of rosaceous plants at two different stages of fruit development were collected from their natural habitats. *Prunus armeniaca*, *Prunus domestica* var. *Kalen-Heikha*, *Prunus persica* var. light pink flower were collected from plants grown near Ladies Hostel, Manipur University complex. *Docynia indica* and *Prunus domestica* var. *Mao-Heikha* were collected from Ukhrul District and two varieties of *Manihot esculenta* Crantz were collected from Thoubal District of Manipur. These plants are selected for the estimation of HCN (Prussic acid content) and other nutritional and anti-nutritional factors in their fruits in two stages, seeds of *phaseolus lunatus* and in *Manihot esculenta* root (Table 1 and Fig. 1).

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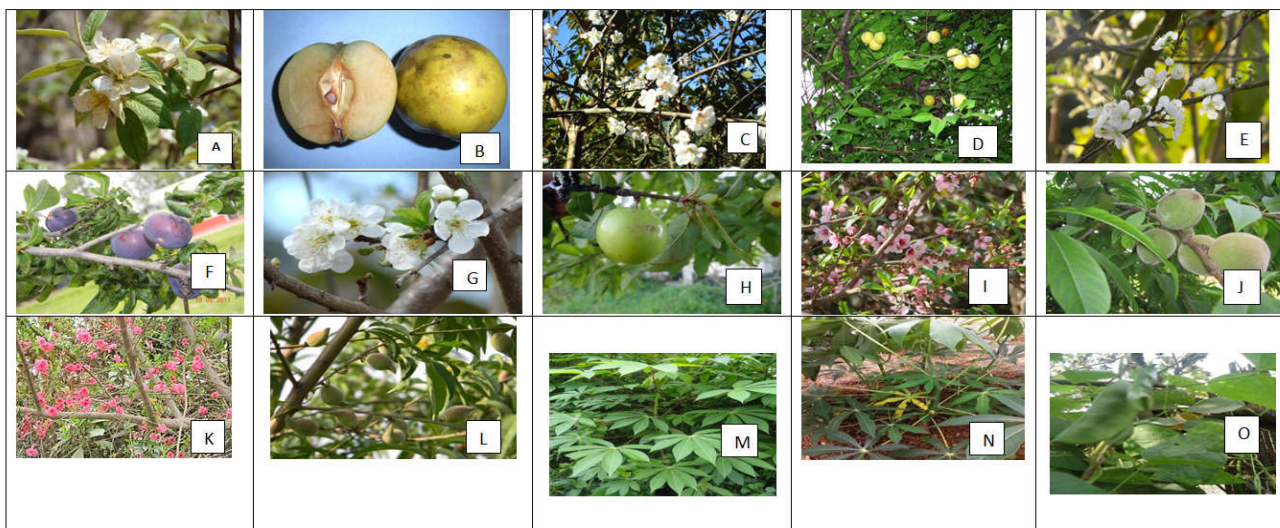


Fig 1 A *Docynia indica* (Wall.) Decne. Flower, B. *Docynia indica* (Wall.)Decne. Fruit, C. *Prunus armeniaca* L. flower, D. *Prunus armeniaca* L. fruit, E. *Prunus domestica* L. var. *Kalen heikha* flower, F. *Prunus domestica* L. var. *Kalenheikha* fruit, G. *Prunus domestica* L. var. *Mao heikha* flower, H. *Prunus domestica* L. var. *Mao heikha* fruit, I. *Prunus persica* (L.) Stokes Var. Light pink flower, J. *Prunus persica* (L.) Stokes Var. Light pink fruit, K. *Prunus persica* (L.) Stokes Var. Deep pink flower, L. *Prunus persica* (L.) Stokes Var. Deep pink fruit, M, *Manihot esculenta* Crantz var. Red cortex, N. *Manihot esculenta* Crantz var. White cortex, O. *Phaseolus lunatus* L.

Estimation for Cyanide Content

Cyanide content was estimated by Bradbury *et al.*(1999) method. Hydrocyanic acid which is evolved from the sample forms a red coloured compound with sodium picrate and the intensity is measured at 625nm. One hundred milligram fresh plant materials were weighed. They are homogenized in 25ml of distilled water by using pestle and mortar. The homogenate was placed in 250ml glass bottle with a few drops of chloroform. The alkaline picrate saturated filter paper was placed inside the glass bottle with the help of a plastic paper stand and tightly covered and incubated at room temperature for 24hr. The sodium picrate in the filter paper was reduced to reddish compound in proportion to the amount of hydrocyanic acid evolved.

Estimation of Saponin

Estimation of Saponin was done following Hiai *et al.* (1976). Vanillin in acidic medium gives chromogens with the absorbance maxima at 544 nm, depending on the nature of the saponins. For extraction of sample, 500mg of defatted sample in a 250ml flask and added 50ml of 50% aqueous methanol. It was kept on a magnetic stirrer overnight at room temperature. The contents were centrifuged at 3000g for 10min and collected the supernatant. Extraction is repeated with the same solvent by stirring on a magnetic stirrer for overnight. After centrifugation, first supernatant with the second one combined together.

Phytic acid

Phytic acid is estimated following the method of Makkar *et al.* (2007). Phytic acid, as a result of possessing negative charge at a wide range of pH values, has strong affinity to bind metal ions such as with calcium, zinc and iron. This leads to interference in the absorption of these minerals from small intestine and adversely affects various metabolic processes. In addition, phytic acid is also known to complex with proteins and starch, resulting in reduced digestibility of these nutrients. The phosphorous in phytic acid is not nutritionally available to monogastric animals.

Nonetheless non-antinutritive concentration of phytic acid in dietary sources is recently considered to be a potential antioxidant. Phytic acid has recently been suggested to have a protective role in carcinogenesis (Makkaret *al.*, 2007).

Table 1 Selected Plants Species for estimation of secondary metabolites along with their local names, locality and their Voucher Number

Species	Local Name	Locality	Voucher Number
<i>Docynia indica</i> (Wall.) Decne.	<i>Heitup</i>	Ukhrul	000296 (MUMPs)
<i>Prunus armeniaca</i> L.	<i>Malhei</i>	ElangKhanpokpi	000291 (MUMPs)
<i>Prunus domestica</i> L. Var <i>Kalen-heikha</i>	<i>Heikha</i>	Thoubal	000293 (MUMPs)
<i>Prunus domestica</i> L. var <i>Mao- heikha</i>	<i>Mao Heikha</i>	Ukhrul	000299 (MUMPs)
<i>Prunus persica</i> (L.) Stokes var. light pink flower	<i>Chumbrei</i>	Canchipur	000294 (MUMPs)
<i>Prunus persica</i> (L.) Stokes var. deep pink flower	<i>Chumbrei</i>	Wangkhei	000295(MUMPs)
<i>Manihot esculenta</i> Crantz var. red cortex	<i>U- Mangra</i>	ElangKhanpokpi	000297(MUMPs)
<i>Manihot esculenta</i> Crantz var. white cortex	<i>U- Mangra</i>	ElangKhanpokpi	000872(MUMPs)
<i>Phaseolus lunatus</i> L.	<i>Hawai-Kalandri</i>	Wangkhei	000873(MUMPs)

Estimation of Total Phenol

Total phenol content was estimated by using Folin-Ciocalteu's reagent (FCR) (Thimmaiah, 1999). Phenols react with an oxidizing agent phosphomolybdate in Folin- Ciocalteu reagent under alkaline conditions and result in the formation of blue coloured complexes, the molybdenum blue is measured at 650nm. Weighed 100mg of powdered dried samples were crushed with 10ml of 80% ethanol using pestle and mortar. The slurry was then centrifuged at 5000rpm for 20min. The supernatants were collected in a test tube. Then the residue is again re-extracted with 5ml of 80% ethanol. The supernatants were collected again and then evaporated in a Petri plate to dryness. Then the dried residue is dissolved in 5ml of distil water. The pellets were discarded and supernatant was made up to 10ml with distil water. 100µl of the dissolved residue

was taken and its volume was made up to 3ml with distilled water. In the test tubes containing test samples, 0.5ml of Folin-Ciocalteu reagent was added. Then after 2 min 20% of Na_2CO_3 were added and mixed thoroughly. The contents were kept in a boiling water bath for about 1 minute. Then the test tubes were cooled in running tap water and absorbances of the blue coloured complex were taken against blank at 650nm with the help of spectrophotometer. The total phenol content was calculated and expressed in mg/g using a standard curve prepared from catechol.

Estimation of Ortho-dihydric Phenols

Ortho-dihydric phenols were estimated using Arnow's reagent (Thimmaiah, 1999). Arnow's reagent specifically reacts with ortho-dihydric phenols and produces a pink coloured complex which is measured calorimetrically at 515nm. Extraction is same as above. 0.5ml of the sample extract was transferred in a test tube and made up to 1.0ml of Arnow's reagent, 8.0ml of water and 2.0ml of 1.0N NaOH were added. It was mixed thoroughly. The absorbance of the pink colour was measured at 515nm. The amount of ortho-dihydric phenol present in the sample was calculated using the standard curve prepared from catechol and expressed as mg/g of fruit tissue.

Estimation of Bound Phenol

The bound phenols liberated by treatment of plant tissues with NaOH at room temperature. The alkali extract contains the released phenols which are measured spectrophotometrically at 290nm. For extraction, 100mg of powder sample was ground with 4ml of SDS solution in a mortar with pestle. The contents were transferred in a tube and centrifuge at 2000rpm for 5 minute. The supernatant was discarded. The residue was washed successively once with 5ml of Sodium lauryl sulphate solution (SDS) solution, twice with 5ml of water, twice with 5ml of ethanol and twice with 5ml of diethyl ether. The supernatants discarded and the residue was dried and preserved. 3ml of 0.5M NaOH was added to the above dry residue and kept at room temperature overnight. Next day, the mixture was centrifuge and the supernatant was saved. 0.5ml of supernatant was transferred into a tube and subjected to the same procedure in total soluble phenol estimation.

Estimation of Total Flavonoid Content

Aluminum Chloride spectrophotometric method was used for flavonoids determination (Chang *et al.*, 2002) with slight modification. For extraction of sample, 100mg of the powdered dried samples were crushed with 10ml of methanol by intermittent maceration up to 48hr. The solvent was evaporated and reduced up to 5ml at room temperature. After evaporation, samples were centrifuged at 10,000rpm for 15 minute at room temperature. The supernatants were collected and volume was made up to 5ml with methanol. 100 μ l of the supernatant was taken and it was added with 100 μ l of aluminum chloride (10%), 0.1ml of potassium acetate (1M) and 2.7ml of distilled water to make volume to 3ml. The reaction mixture was kept at room temperature for 30min. The absorbance was measured at 415nm using spectrophotometer. The calibration curve was prepared using different concentrations of quercetin expressed in mg/gm dry weight.

Estimation of Tannins

The tannins are estimated by Folin Denis method which is based on the non-stoichiometric oxidation of the molecules containing a phenolic hydroxyl group (Thimmaiah, 1999). Tannin like compounds reduces phosphomolybdic acid in alkaline solution to produce a highly coloured blue solution, the intensity of which is proportional to the amount of tannins. The intensity is measured in a spectrophotometer at 700nm. For extraction of plant samples, 100mg of the dried powdered samples were weighed and kept in magnetic stirrer for 3hrs after adding 10ml of 80% ethanol. The extracts were centrifuged for 15 minute at 10,000rpm. The supernatants were collected and stored for further analysis. From the collected supernatants, 0.1ml of sample of each sample was added and 7.5ml of distilled water were added. After that, 0.5ml of Folin Denis Reagent (FDR) followed by 1ml of 35% Na_2CO_3 . The final volume is made up to 10ml with distilled water. The blue colour appeared is measured at 700nm by using UV-VIS Beam spectrophotometer. The calibration curve was prepared using tannic acid expressed in mg/g dry weight.

Statistical Analysis

The results of biochemical studies were provided as mean \pm SEM and they are analyzed for their significance difference by using one way analysis of variance (ANOVA) test followed by Tukey's significant difference post hoc comparison. Pearson Correlation values among the parameters taken up for biochemical analysis were performed using SPSS version 15 and their values were presented in the form of a table.

RESULTS AND DISCUSSION

The toxicity of HCN, a role in plant protection against herbivores, pathogens, and competitors is appealing. Indeed it favours a defence function against certain animals including insects (Jones, 1988 and Nahrstedt, 1985). Species showing cyanogenic polymorphism have frequently been exploited to investigate such plant-herbivore interactions. Cyanogenic glycosides are constitutive defence compounds found in about 5% of all plants (Jones, 1998; Gleadow and Woodrow, 2002). When plant tissue containing cyanogenic glycosides is crushed or chewed, the glycosides are mixed with endogenous α -glucosidases, and toxic hydrogen cyanide (HCN) is released.

From Table 2, it is observed that the HCN content in the different parts of the selected plant species of Rosaceae family. In the flower of selected Rosaceae species, maximum HCN content was found in the *P. persica* (L.) Stokes var. Deep pink flower (1.30 ± 0.183 mg/g FW). In the leaves of selected Rosaceae family, maximum HCN was found in the *P. persica* (L.) Stokes var. light pink flower leaf (1.56 ± 0.075 mg/g FW). In pre mature seed of selected Rosaceae species, HCN is found to be maximum in *P. armeniaca* seed (3.27 ± 0.155 mg/g FW) and minimum in *P. domestica* var. *Kalen heikha* (0.89 ± 0.091 mg/g FW).

In mature seed of selected Rosaceae species, HCN was found to be maximum in *P. persica* (L.) Stokes var. light pink flower (1.79 ± 0.026 mg/g FW) and minimum in *P. persica* (L.) Stokes var. deep pink flower (1.47 ± 0.087 mg/g FW). HCN content in the selected 9 (nine) plants was maximum in *Phaseolus lunatus* seed (2.56 ± 0.134 mg/g FW) and minimum in leaf of *P. armeniaca* (0.053 ± 0.007 mg/g FW). Even though the content of HCN is minimum in *Manihot esculenta* var. red cortex (1.07 ± 0.013 mg/g FW) and *Manihot*

esculenta var.white cortex (0.73±0.139mg/g FW) respectively may also cause toxic to the consumers (Table 2).

Table 2 Cyanide content in nine edible plant parts (mg/g FW ± SEM)*

Species	Flower	Leaf	Premature seed	Mature seed	Root
<i>Docynia indica</i> (Wall.) Decne.	N.D	N.D	1.11 ± 0.156	1.54 ± 0.081	-
<i>Prunus armeniaca</i> L.	0.81 ± 0.246	0.05 ± 0.007	3.27 ± 0.155	1.73 ± 0.139	-
<i>Prunus domestica</i> L. var. <i>Kalen heikha</i>	0.30 ± 0.089	0.09 ± 0.017	0.89 ± 0.091	1.71 ± 0.173	-
<i>Prunus domestica</i> L. Var. <i>Mao Heikha</i>	0.15 ± 0.015	0.14 ± 0.025	1.55± 0.097	1.48 ± 0.128	-
<i>Prunus persica</i> (L.) Stokes var. light pink flower	0.69 ± 0.085	1.56 ± 0.075	1.54 ± 0.197	1.79 ± 0.026	-
<i>Prunus persica</i> (L.) Stokes var. deep pink flower	1.30 ± 0.183	0.93 ± 0.101	1.35± 0.291	1.47 ± 0.087	-
<i>Manihot esculenta</i> var. red cortex	-	-	-	-	1.07 ^a ± 0.013
<i>Manihot esculenta</i> var. white cortex	-	-	-	-	0.73 ^b ± 0.139
<i>Phaseolus lunatus</i> seed	-	-	-	2.56 ^c ±0.134	-

*Data presented as mean ± SEM. Data were analysed by ANOVA and within each row different letters indicate statistically different values according to post-hoc comparison (Tukey HSD) at P<0.05. N.D. = Not detected.

Soto-Blanco and Górnjak (2010) reported that the ingestion of leaves and roots by goats can be attributed to the action of cyanide released from cyanogenic glycosides in consecutive days. Toxic cyanide is released when the plant cut into small pieces during food preparation and the resulting hydrogen cyanide is easily removed by cooking in water since it is volatile (Harbone, 1972). Accumulation of cyanogenic glucosides in certain angiosperm seeds provide a storage deposit of reduced nitrogen and sugar for the developing seedling (Liberei *et al.*, 1985). Different species plant present different cyanogenic glucosides like Cassava (*Manihot esculenta*), barley (*Hordicum vulgare*) (Andersen *et al.*, 2000; Forslund and Johnson 1997; Neilsen *et al.*, 2002).

Saponin content in the selected nine samples was found to be maximum in *Phaseolus lunatus* seed (8.92 ± 0.466 mg/100g) and minimum in pre-mature fruit of Rosaceae, *P. domestica* var. *Mao Heikha* (0.37 ± 0.041 mg/100g) [Table 3]. Saponin content in premature fruit of Rosaceae was found to be maximum in *P. persica* var. deep pink flower (0.86 ± 0.131 mg/100g DW) and minimum in *P. domestica* var. *Mao Heikha* (0.37 ± 0.041 mg/100g DW). In mature fruits of Rosaceae, saponin content was found to be maximum in *Docynia indica* (6.10 ± 0.360 mg/100g DW) and minimum *P. persica* var. light pink flower (1.00 ± 0.082mg/100g DW)[Table 2]. In seed of Rosaceae family, highest saponin content was found in the *P. persica* var. deep pink flower seed (6.42 ± 0.109 mg/100g DW) and lowest content was found in the *P. domestica* var. *Mao Heikha* (3.46 ± 0.202 mg/100g DW). Among the two varieties of *Manihot esculenta*, highest content of saponin was found in *Manihot esculenta* var. white cortex (6.44 ± 0.102 mg/100g DW)[Table 3].

The presence of saponin in the selected nine samples is a common occurrence even in a wide variety of food plants viz.,

Bengal gram, soybean, navy beans, haricot beans and kidney beans being relatively rich, (Fenwick and Oakenfull, 1983; Oakenfull and Sindhu, 1990). Consumption of these selected nine samples may cause abdominal pain, vomiting and diarrhoea. However, saponins are also important in human diet as they reduce the risk of heart diseases. It has also been reported (Applebaum *et al.*, 1969) that they induce resistance among legume seeds against insect attack. In epidemiological studies, saponins have been shown to have an inverse relationship with the incidence of renal stones (Shi *et al.*, 2004).

Non-antinutritive concentration of phytic acid in dietary sources is recently considered to be a potential antioxidant. Phytic acid has recently been suggested to have a protective role in carcinogenesis (Makkar *et al.*, 2007). Phytic acid content in selected nine samples, maximum content of phytic acid was found in the *Phaseolus lunatus* seed (16.18 ± 0.993 mg/100g DW) and minimum in pre-mature *Prunus armeniaca* (1.58 ± 0.043mg/100g DW) fruit. Phytic acids content in premature fruits of Rosaceae were shown in Table 3.

In premature fruits Rosaceae, highest phytic acids content was found in the *Docynia indica* (2.25 ± 0.098mg/100g DW) and minimum in *Prunus armeniaca* (1.58 ± 0.043mg/100g DW). In mature fruits or Rosaceae, maximum contents of phytic acid were also found in the *Docynia inidca* (1.81 ± 0.033mg/100gDW) and minimum in *Prunus armeniaca* (0.56 ± 0.015mg/100gDW) [Table 3]. In selected seed of Rosaceae family, maximum phytic acid content is found in the seed of *Prunus armeniaca* (4.90 ± 0.055 mg/100g DW) and minimum in *Docynia indica* seed (1.85 ± 0.113mg/100g DW) [Table 3].

The phenolic compounds are groups of compounds containing a benzene ring with one or more hydroxyl groups which includes flavonoids, tannin etc.

Table 3 Phytic acids (PA) and Saponin(SA) contents in pre-mature fruit (PF),mature fruit (MF), seeds (SD) and roots (RT)of nine edible plant parts (mg/100g DW ± SEM)

Plant	<i>Docynia indica</i> (Wall.) Decne.			<i>Prunus armeniaca</i> L.			<i>Prunus domestica</i> L. var. <i>Kalen Heikha</i>			<i>Prunus domestica</i> L. var. <i>Mao Heikha</i>			<i>Prunus persica</i> (L.) Stokes var. light pink flower			<i>Prunus persica</i> (L.) Stokes var. deep pink flower			<i>Manihot esculenta</i> var. red cortex		<i>Manihot esculenta</i> var. white cortex		<i>Phaseolus lunatus</i>
	PF	MF	SD	PF	MF	SD	PF	MF	SD	PF	MF	SD	PF	MF	SD	PF	MF	SD	RT	RT	SD		
PA	2.25 ^a ± 0.098	1.81 ^a ± 0.033	1.85 ^a ± 0.113	1.58 ^b ± 0.043	0.56 ^a ± 0.015	4.90 ^b ± 0.055	2.17 ^a ± 0.064	0.85 ^c ± 0.028	4.1 ^c ± 0.103	2.13 ^a ± 0.064	0.72 ^{bc} ± 0.034	2.48 ^d ± 0.034	1.64 ^b ± 0.023	0.70 ^{bc} ± 0.028	2.06 ^a ± 0.063	1.63 ^b ± 0.026	0.73 ^{bc} ± 0.026	2.80 ^d ± 0.095	0.68 ^a ± 0.102	0.97 ^b ± 0.032	16.18 ^c ± 0.993		
SA	0.84 ^a ± 0.090	± 0.360	± 0.066	± 0.022	± 0.065	± 0.250	± 0.008	± 0.008	± 0.184	± 0.041	± 0.040	± 0.202	± 0.042	± 0.082	± 0.131	± 0.098	± 0.109	± 0.185	± 0.185	± 0.102	± 0.466		

Phenols are water soluble substances and with sugars many forms glycosides, thus located in the cell vacuoles. These compounds play an important role in the precursor of toxic substances and role in the growth regulation and development of plants. The tannins, flavonoids and other phenolic compounds play major role in preventing number of chronic diseases like anti-inflammatory, anti-thrombic, antioxidant and anti-carcinogenic activities (Craig, 1999). Tannins are considered as anti-nutritional factor, their beneficial or anti-nutritional properties depend upon their structure and dosages. Introduction of more hydrophilic groups (-COOH) or more lipophilic (aromatic) groups will affect the solubility thereby influencing the orientation of the molecule and consequently toxic action (Mahadevan, 1982). Tannin also complexes with proteins, divalent metals, cellulose, hemicellulose, pectin and other carbohydrates (Mahanato *et al.*, 1982). High consumption of tannin is dangerous to health, being a phenolic secondary metabolites with one or more hydroxyl substituents bonded to aromatic ring, it produces anthocyanides, another toxic product an acid degradation (Gatachewet *et al.*, 2000; Waterman and Cole, 1994).

Phenolic compounds are common constituents of many different plants. A wide variety of phenolic compounds have been reported in plants in which exist both in single and complex forms. Phenolic compounds are secondary metabolites which have been associated with flavour and colour characteristics of fruits and vegetables and are gaining attention because of their potent antioxidant and health promoting properties (Kaur and Kapoor, 2001).

Fig. 2 presented the Phenolic compounds content in premature fruits of Rosaceae. The total phenol content is maximum in *Prunus persica* var. deep pink flower (29.67±0.321mg/g DW) and minimum in *Docynia indica* (18.22±0.752mg/g DW). Maximum tannin content is found in *Docynia indica* (32.29±0.313mg/g DW) and minimum in *Prunus domestica* var. *Kalen Heikha* (18.62±0.710mg/g DW). In premature fruits, flavonoid content is found to be maximum in *P. armeniaca* (0.49±0.075mg/g DW) and minimum in *Prunus persica* (L.) Stokes var. light pink flower 0.25±0.010mg/g DW).

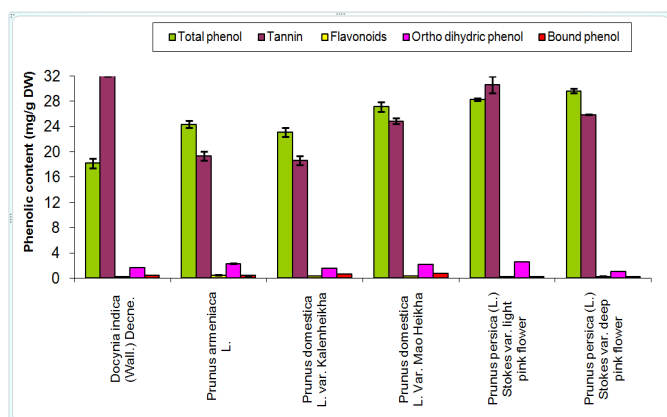


Fig 2 Phenolic compound content in pre-mature fruits of Rosaceae family

Highest content of ortho-dihydric phenol is found in *P. persica* var. light pink flower (2.60±0.098mg/g DW) and lowest in *P. persica* var. deep pink flower (1.04±0.004mg/g DW). Bound phenols content is maximum in *P. domestica* var. *Kalen*

Heikha (0.76 ± 0.033mg/g DW) and minimum in *P. persicavar.* deep pink flower (0.24 ± 0.014mg/g DW). Fruits and cereals are main dietary sources of polyphenols which are major antioxidant of common constituents of food of plant origin. Intakes of chocolate and dry legums are contributed to polyphenol intake (Scalbert *et al.*, 2005).

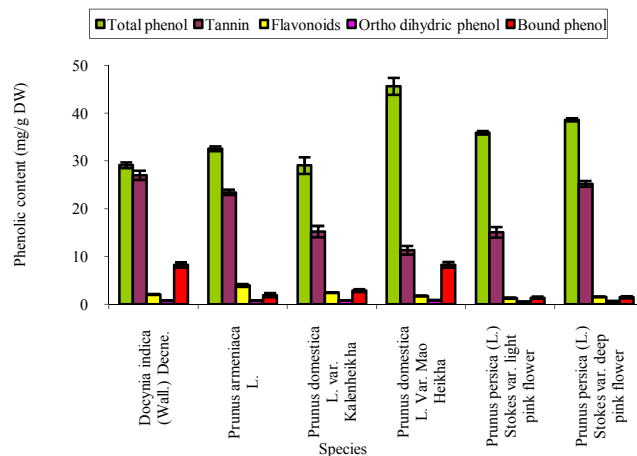


Fig 3 Phenolic compound content in mature fruits of Rosaceae family

Fig. 3 shows the Phenolic compound contents in the selected mature fruits under Rosaceae family. The total phenol contents is in maximum *Prunus domestica* L. var. *Mao Heikha* in (45.63 ± 1.781mg/g DW) and minimum in *Prunus domestica* L. var. *KalenHeikha* (29.06 ± 1.741mg/g DW). Tannins content in mature fruits of Rosaceae family were found to be maximum in *Docynia indica* (27.01 ± 0.953mg/g DW) and minimum in *Prunus domestica* var. *Mao Heikha* (11.34 ± 0.902mg/g DW). Flavonoid content in mature fruits was found to be highest in *Prunus armeniaca* (3.95 ± 0.275mg/g DW) and minimum in *Prunus persica* (L.) stokes var. light pink flower (1.30 ± 0.102). Ortho - dihydric phenol content in selected mature fruits of Rosaceae is maximum in *P. domestica* var. *Mao Heikha* (0.89 ± 0.035mg/g DW) and minimum in *P. persica* (L.) Stokes var. *light pink flower* (0.52 ± 0.025 mg/g DW). Bound phenols content in selected mature fruits of Rosaceae was found to be maximum in *P. domestica* var. *Mao Heikha* (8.27 ± 0.571mg/g DW) and minimum in *P. persica* (L.) Stokes var. *light pink flower* (1.39 ± 0.216mg/g DW).

In Table 4, amongst the seeds of selected Rosaceae fruits, total phenol content is maximum in *P. persica*(L.) Stokes var. light pink flower (0.32 ± 0.006 mg/g DW) and minimum in *P. domestica* L. var. *Mao Heikha* (0.01 ± 0.009 mg/g DW). Tannin content is maximum in *P. armeniaca* seed (0.61 ± 0.182 mg/g DW) and minimum in *P. domestica* L. var. *Mao Heikha* (0.36 ± 0.069 mg/g DW). Ortho dihydric phenol content in selected Rosaceous seeds was found to be maximum in *P. domestica* L. var. *Mao Heikha* (1.82 ± 0.076 mg/g DW) and minimum in *P. persica* stokes var. light pink flower (0.44 ± 0.189 mg/g DW). Bound phenol content in selected Rosaceous seeds was maximum in *P. domestica* L. var. *Mao Heikha* (4.25 ± 0.433 mg/g DW) and minimum in *P. domestica* L. var. *Kalen Heikha* (0.37 ± 0.038 mg/g DW) [Table 4]. Between the two selected varieties of *Manihot esculenta* Crantz.the variety white cortex has more total phenol (6.11 ± 1.753 mg/g DW) with the red cortex has minimum

total phenol (3.64 ± 0.575 mg/g DW). The total phenol content in *Phaseolus lunatus* seed was 18.09 ± 0.523 mg/g DW (Table 5).

Table 4 Phenolic compounds in mature seeds of Rosaceae family (mg/g DW \pm SEM)

Species	Total phenol	Tannin	Ortho dihydric phenol	Bound phenol
<i>Docynia indica</i> (Wall.) Decne.	$0.03^a \pm 0.005$	0.48 ± 0.196	$0.63^a \pm 0.164$	$0.84^a \pm 0.042$
<i>Prunus armeniaca</i> L.	$0.13^b \pm 0.016$	0.61 ± 0.182	$1.23^b \pm 0.153$	$1.26^b \pm 0.128$
<i>Prunus domestica</i> L. var. <i>Kalen heikha</i>	$0.13^b \pm 0.011$	0.37 ± 0.103	$0.62^a \pm 0.095$	$0.37^a \pm 0.38$
<i>Prunus domestica</i> L. Var. <i>Mao Heikha</i>	$0.01^a \pm 0.009$	0.36 ± 0.069	$1.82^c \pm 0.076$	$4.25^c \pm 0.433$
<i>Prunus persica</i> (L.) Stokes var. light pink flower	$0.32^c \pm 0.006$	0.50 ± 0.198	$0.44^a \pm 0.189$	$0.56^a \pm 0.056$
<i>Prunus persica</i> (L.) Stokes var. deep pink flower	$0.07^a \pm 0.009$	0.42 ± 0.016	$1.41^b \pm 0.133$	$2.84^d \pm 0.289$

*Data presented as mean \pm SEM. Data were analysed by ANOVA and within each column different letters indicate statistically different values according to post-hoc comparison (Tukey HSD) at $P < 0.05$.

Table 5 Different secondary metabolites content in selected plants of Manipur

Species	Cyanide (mg/g)	Total phenol (mg/g)	Bound phenol (mg/g)	Ortho - phenol (mg/g)	Tannin (mg/g)	Saponin (mg/100g)	Phytate (mg/100g)
<i>Manihot esculenta</i> var. red cortex	$1.07^a \pm 0.013$	$3.64^a \pm 0.575$	$0.018^a \pm 0.001$	$1.74^a \pm 0.167$	$0.22^a \pm 0.062$	$5.69^a \pm 0.185$	$0.68^a \pm 0.102$
<i>Manihot esculenta</i> var. white cortex	$0.73^b \pm 0.139$	$6.11^b \pm 1.753$	$0.01^a \pm 0.001$	$2.03^b \pm 0.065$	$0.41^a \pm 0.038$	$6.44^b \pm 0.102$	$0.97^b \pm 0.032$
<i>Phaseolus lunatus</i> seed	$2.56^c \pm 0.134$	$18.09^c \pm 0.523$	$0.23^a \pm 0.008$	$2.18^b \pm 0.151$	$5.93^b \pm 0.131$	$8.92^c \pm 0.466$	$16.18^c \pm 0.993$

*Data presented as mean \pm SEM. Data were analysed by ANOVA and within each row different letters indicate statistically different values according to post-hoc comparison (Tukey HSD) at $P < 0.05$.

CONCLUSION

Considerable amount of plant metabolites like total phenols, tannins, and flavonoids are present in the selected nine plants viz. Among the fruits of Rosaceae family which is consumed in large amounts in Manipur, prussic acids are present only in the seed. If we do not consume the seed, the toxic effect can be avoided. Generally, *Phaseolus lunatus* seed is taken in cooked form. Cooking reduces the quantity of prussic acid. Fresh root of *Manihot esculenta* contains high amount of HCN, it is necessary to cook the root for safe consumption. Devi and Singh (2013) reported that Vitamin C is more responsible for free radical scavenging activity and different phenolic compounds with their antioxidant activity in selected rosaceous fruits. Among the nine selected plant for biochemical analysis, no plant showed an overall maximum biochemical contents as a whole. Human body can get mineral elements from plant sources (WHO, 1996). Evaluation of nutritive and anti-nutritional value of these selected samples provides an easily accessible natural source that could be used as food supplement. Though all the analysed fruits contains saponins, phytates and tannins, it is found that the edible pulp of the six fruits namely *Docynia indica*, *Prunus armeniaca*, *P. domestica*, *P. persica* are useful for consumption and the seed of these six fruits were dangerous to health if taken at a large quantity. Fresh consumption of

Manihot esculenta root in large quantity is dangerous to human being.

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References

- Andersen, MD., Busk, P.K., Svendsen, I. and Moller B.L. 2000. Cytochromes P-450 from cassava (*Manihot esculenta* crantz) catalyzing the first steps in the biosynthesis of the cyanogenic glucosides linamarin and lotaustralin: Cloning, functional expression in pichipastories and substrate specificity of the isolated recombinant enzymes. *J. Biol. Chem.* 275: 1966-1975.
- Applebaum, S.W., Marco, S. and Birk, Y. 1969. Saponin as possible factors of resistance of legume seeds to the attack of insects. *J. Agric. Food Chem.* 17: 618-620.
- Bradbury, M.G., Egan, S.V. and Bradbury, J.H. 1999. Determination of all forms of cyanogens in cassava roots and cassava products using picrate paper kits. *Journal of the Science of Food Agriculture*, 79: 593-601.
- Craig, W. J. 1999. Health-promoting properties of common herbs. *Am. J. Clin. Nutr.* 70: 4915-4998.
- Croteau, R., Kutchan, T.M. and Lewis, N.G. 2000. Natural products (Secondary metabolites). In: *Biochemistry & Molecular Biology of Plants* (eds. B. Buchanan, W. Gruissen & R. Jones), pp. 1250-1318. *American Society of Plant Physiologist*, Rockville, Maryland.
- Dacie, J.V. and Lewis S.M. 1984. *Practical haematology*. 6th ed. London: Churchill Living stone.
- Devi, L.K. and Singh, P.K. 2013. Proximate Composition of Phenolic Compounds and Vitamin C with Potential Antioxidant activity in selected Rosaceae fruits of Manipur. *NeBio*, 4(5): 33-38, *ISSN* 2278-2281 (Online Version) *ISSN*: 0976-3597 (Print Version).
- Fenwick, D.E. and Oakenfull, D. 1983. Saponins content of food plants and some prepared foods. *Journal of the Science of Food and Agriculture*, London. 34(2): 186-189.
- Forslund, K. and Johnson, L. 1997. Cyanogenic glycosides and their metabolic enzymes in barley, in relation to nitrogen levels. *Physiol Plant.* 101: 367-372.
- Franks, T.K., Hayasaka Y., Choimes S. and Van Heeswijck, R. 2005. Cyanogenic glucosides in grapevine: polymorphism, identification and developmental patterns. *Phytochemistry*. 66: 165- 173.
- Gatechew, G., Makkar, H. and Becker, K. 2000. Effects of polyethylene glycol on in vitro degradability of nitrogen from tannin rich browse and herbaceous legumes. *Brit J Nutri.* 84: 73-83.
- Gleadow, R.M., and Woodrow, I. E. 2002. Constraints on effectiveness of cyanogenic glycosides in herbivores defence. *J. Chem. Ecol.* 847-858.
- Gurr, E. 1953. *A practical manual of medical and biological techniques*. Leonard Hill Ltd, London.

- Harbone, J.B. 1972. *Cyanogenic glucosides and their function*. In: *Phytochemical ecology*. London Academic Press. p. 104-123.
- Hiai S., Oura, H. and Nakajima, T. 1976. Color reaction of some saponins and saponins with vanillin and sulphuric acid. *Planta Medica* 29, 116- 122.
- Jones, D.A. 1988. Cyanogenesis in animal-plant interactions. In D. Evered, S. Harnett, eds., *Cyanide Compounds in Biology*, Ciba Foundation Symposium No 140. John Wiley & Sons.
- Jones, D.A. 1998. Why are so many plants cyanogenic? *Phytochemistry*, 37: 477-492.
- Kaur, C. and Kapoor, H.C. 2001. Antioxidants in fruits and vegetables the millennium's health: *International journal of Food Science and Technology*: 36, 703-725.
- Lieberei, R., Selmar, D. and Biehl, B. 1985. Metabolization of cyanogenic glucosides in Hevea brasiliensis. *Plant SystEvol*, 150: 49-50.
- Mahadevan, A. 1982. Biochemical aspects of Plant Disease Resistance (Part 1: *Preformed inhibitory substances "Prohibitions"*). R.K Jain (eds.), Karol Bagh, New Delhi.
- Mahanato, S.B., Ganguly, A.N. and Sadhu, N.P. 1982. Review- Steroid saponins. *Phytochemistry*, 21(5): 959-978.
- Makkar, H.P.S., Sidduraju, P. and Becker, K. 2007. *Plant Secondary metabolites*. Humana Press, Totowa, New Jersey.
- Mayer, A.M. 1987. Polyphenol oxidase in plants-recent progress. *Phytochemistry*, 26:11-20.
- Montilla, E.C., Hillbrand, S., Antizana, A. and Winterhalter, P. 2010. Soluble and bound phenolic compounds in different Bolivian purple Corn (*Zea mays* L.) cultivars. *Agriculture Food Chemistry*, 59: 7068-7074.
- Nahrstedt, A. 1985. Cyanogenic compounds as protecting agents for organisms. *Plant SystEvo*. 150: 35-47.
- Nardini, M., Cirillo, E., Natella, F. Maencarelli, D., Comisso, A. and Scaccini, C. 2002. Detection of bound phenolic acids: Prevention by ascorbic acid and ethylene diaminetetraacetic acid of degradation of phenolic acids during alkaline hydrolysis. *Food Chemistry*, Volume 79. Issue I, 119-124.
- Neilsen, L.A., Dsen C.E., Pantoppidan, K. and Moller. 2002. Leucine derived cyanoglycosides in barley. *Plant physiol*. 129: 1066-1075.
- Oakenfull, D. G. and Sindhu, G. S. 1990. Could saponins be a useful treatment for hypercholesterolaemia? *Eur. J. Clin. Nutr.*, 44, 79-88.
- Ologunde, M.O., Olaniyan, S.A., Fapojuwo, O.O., Liasu, M.O. and Olunlade, B.A. 2008. Haematological parameters on rats fed on prolonged crude oil contaminated cassava-based diet. *American - Eurasian Journal of Sustainable Agriculture*. 2(3): 242- 248.
- Scalbert, A., Manah, C. and Morand, C. 2005. Dietary polyphenols and the prevention of diseases. *Crit. Rev. Food Sic., Nutr.* 45, 285-306.
- Shi, J., Arunasalam, K., Yeung, D., Kakuda, Y., Mittal, G. and Jiang, Y. 2004. Saponins from edible legumes: chemistry, processing and health benefits. *J Med Food*. 7(1):67-78.
- Soto-Blanco, B. and Górnjak, S.L. 2010. Toxic effects of prolonged administration of leaves of cassava (*Manihotesculenta* Crantz) to goats. *Exp Toxicol Pathol*. 62(4):361-366. doi: 10.1016/j.etp.
- Thimmaiah, S.R. 1999. *Standard Methods Biochemical Analysis*. Kalyani Publishers New Delhi-110002.
- Vetter, J. 2000. Plant cyanogenic glycosides. *Toxicon*, 38: 11- 36.
- Waterman, P.G. and Cole, S. 1994. In: *Analysis of phenolic plant metabolites*, London: Blackwell Scientific Publications.

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