CHAPTER VII

Study of Anti-nutritional Factors of Wild Fruits

In addition to important nutrients and bioactive compounds, anti-nutrients are also naturally occurring organic chemical compounds found in plants which in excess intake of these play some adverse roles [1]. They reduce the maximum utilization of nutrients especially vitamins, minerals and proteins and thus prevent the optimal exploitation of the nutrients present in a food and decrease the nutritive value. Anti-nutrients are not always harmful even though they lack of nutritive values [2]. Anti-nutrients are beneficial for human health if consumed at appropriate amounts [3], and can prevent diseases including coronary diseases and cancers [4]. Phytate is an example of anti-nutrient which forms insoluble complexes with iron, calcium, zinc and copper [5]. Proteins such as lectins and trypsin inhibitor found in legumes can act as anti-nutrients [6]. Flavonoids are also a form of anti-nutritional factors. These compounds chelate metals such as zinc and iron and reduce the absorption of these nutrients. They also inhibit digestive enzymes and precipitate proteins [7]. Several anti-nutritional factors such as phytic acid, lectins, tannins and amylase inhibitors may lower the rate of starch digestion and hence blood glucose response by the same mechanisms which make them anti-nutrients [8–12]. Saponins which are steroid or triterpenoid glycosides are characterized by a bitter taste and possess a strong haemolytic activity [13].

In this study, the anti-nutritional factors of five wild fruits *viz. Grewia sapida, Eugenia operculata, Antidesma bunius, Ottelia alismoides,* and *Aporosa dioica* from Assam of North East India were investigated and reported.

VII.1 Materials and Methods

VII.1.1 Materials

Five wild edible fruits *viz. Grewia sapida, Eugenia operculata, Antidesma bunius, Ottelia alismoides,* and *Aporosa dioica* mentioned in **Table II.1** were collected from Chirang and Kokrajhar district of Assam, North East India. Phytic acid, vanillin and catechin were obtained from Sigma Aldrich, Bangalore, India. All other reagents used for analysis were analytical grade.

VII.1.2 Sample preparation

The samples of five wild fruits for this study were prepared as per the procedure mentioned in the **Section II.2.3** (**Page no. 67**).

VII.1.3 Determination of oxalate

Total oxalate was determined by titration method described by Day and Underwood [14]. Briefly, 1 g of the sample was weighed into 100 mL conical flask and 75 mL of 3 N H_2SO_4 was added, stirred in a magnetic stirrer for 1 h and then filtered using Whatman No.1 filter paper. The filtrate (25 mL) was taken, heated to 80–90°C and titrated against 0.05 M $KMnO_4$ solution until a faint pink colour appeared that persisted for 30 second. Oxalic acid was then calculated by taking 1 mL of 0.05 M $KMnO_4$ as equivalent to 2.25 mg anhydrous oxalic acid.

VII.1.4 Determination of tannin

Quantitative estimation of tannin as catechin equivalent was carried out using the modified vanillin-HCl method of Price *et al*. [15]. One gram of dry sample was extracted with 10 mL methanol for 24 h, vortexed and then filtered using Whatman No. 1 filter paper and diluted to 25 mL. To 1 mL of extract, 5 mL of reagent mixture (1:1 of 4% vanillin in methanol and 8% concentrated HCl in methanol) was added. After 20 min, the absorbance was read at 500 nm using UV visible Spectrophotometer (Lambda 35, Perkin Elmer, USA) by using different concentration of catechin (10–300 µg/mL) as standard. The results are calculated from the equation of standard graph (**Fig. VII.1**, $y = 0.0009x + 0.0979$, $R^2 = 0.9821$) and expressed in mg/g of dried sample.

VII.1.5 Determination of phytate

Phytate content was determined according to method described by Vaintraub and Lapteva [16]. Briefly, 5 g of dried sample was extracted with 100 mL HCl (2.4%) at ambient temperature for 1 h and centrifuged at 3000 rpm for 30 min. The clear supernatant was collected and used for phytate estimation. To 3 mL of the sample solution, 1 mL of Wade reagent (0.03% solution of $FeC1_3.6H_2O$ containing 0.3% sulfosalicylic acid in water) was added, the mixtures were vortexed and centrifuged at 4000 rpm for 10 min. The supernatant was collected and absorbance was measured at 500 nm using UV visible spectrophotometer (Lambda 35, Perkin Elmer, USA). The phytate concentration was calculated from the difference between the absorbance of the control (3 mL of water + 1 mL Wade reagent) and

that of the sample or standard. For phytic acid standard curve, a series of solution were prepared containing 5–100 μg/mL phytic acid in water and the volume was adjusted to 3 mL with distilled water. To each tube, 1 mL of the Wade reagent was added and the solution was mixed using vortex for 5 sec. The mixture was centrifuged at 4000 rpm for 10 min and the supernatant was read at 500 nm using distilled water as a blank. The phytate was calculated from the phytic acid standard curve equation (**Fig. VII.2**, $y = 0.0003x + 0.0036$, $R^2 = 0.9549$**)** and the results were expressed in mg/g dry weight.

VII.1.6 Determination of saponin

Saponin content was determined according to the method described by Obadoni and Ochuko [17]. 10 g of each dry sample powder was extracted in 100 mL of 20% ethanol and heated over a hot water bath for 4 h with continuous stirring at 55°C. The mixture was filtered using Whatman No. 1 filter paper and the process was repeated twice and the total volume was reduced to 40 mL by evaporating in hot water bath. The extract was then transferred to separating funnel and 20 mL of diethyl ether was added and shaken vigorously. The aqueous layer was collected and the purification process was repeated. After that 60 mL of *n*-butanol was added and washed twice with 10 mL of 5% aqueous sodium chloride solution. The remaining solution was then transferred into the pre-weighted beaker and it was completely dried in hot air oven for constant weight. After drying in hot air oven, it was then cooled in a desiccator and saponin content was calculated with the following formula and the results were expressed in mg/g of dried sample.

$$\text{Saponin content (g)} = \frac{W_2 - W_1}{\text{Weight of the Sample}} \times 100$$

Where, W_1- Initial weight of container (g)

W_2- Final weight of container (g)

VII.1.7 Determination of alkaloid

Alkaloid content was determined using the method of Griffiths [18]. To 5 g of dry sample, 50 mL of 10% acetic acid in ethanol was added and allowed to stand for 4 h and filtered. The filtrate was evaporated to one fourth of its original volume and precipitates were filtered with pre-weighted Whatman No.1 filter paper and washed twice with 1% NH_4OH solution. The precipitates were then dried at 60°C, cooled in a desiccator and weighed until

the constant weight was obtained. It was then calculated by the following formula and the results were expressed in mg/g of dried sample.

$$\text{Total alkaloid (g)} = \frac{W_2 - W_1}{\text{Weight of the Sample}} \times 100$$

Where, W_1 = Initial weight of filter paper.

W_2 = Final weight of filter paper.

VII.1.8 Statistical analysis

All the experiments were carried out for three independent replicates and the data were represented in terms of mean ± standard deviation. OriginPro 8.5 software (MA 01060, OriginLab Corporation, USA) was used for statistical analysis and executed by the one-way ANOVA and *t*-test at $p < 0.05$.

VII.2 Results and Discussion

The standard curves of tannin and phytic acid are shown in **Fig. VII.1** and **Fig. VII.2,** respectively. The anti-nutritional contents of the five wild fruits are presented in the **Table VII.1**. The **Fig. VII.3** shows the variation of anti-nutritional contents in mg/g dried sample of five wild edible fruits. The value of tannin in the test samples ranged from 0.18 ± 0.03 mg/g in *E. operculata* to 1.03 ± 0.01 mg/g in *O. alismoides* which are comparable to the values reported by [19] and these results are lower than the values observed in the fruits (7.5 mg/g) reported by Kozioc and Marcia [20]. The tannin content of these fruits are higher than the tannin content of *Spondias mombin* (2.41 ± 0.02 mg/100 g) and *Mordii whytii* (1.55 ± 0.02 mg/100 g) reported by Adepoju [21]. Umaru *et al*. [22] also studied tannin contents of some wild edible fruits of Northern Nigeria and reported the highest tannin in *Balanite aegyptiaca* (7.40 ± 0.14%) and the lowest in *Parkia biglobosa* (0.93 ± 0.11%) and *Phoenix dactylifera* (0.93 ± 0.215). These values are much lower in comparison to the results of the fruits studied herein. Tannins and alkaloids are well known to possess antimicrobial, anthelmintic and anti-diarrhoeal activities [23]. Tannins have been reported to possess astringent properties that hasten the healing of wounds [24].

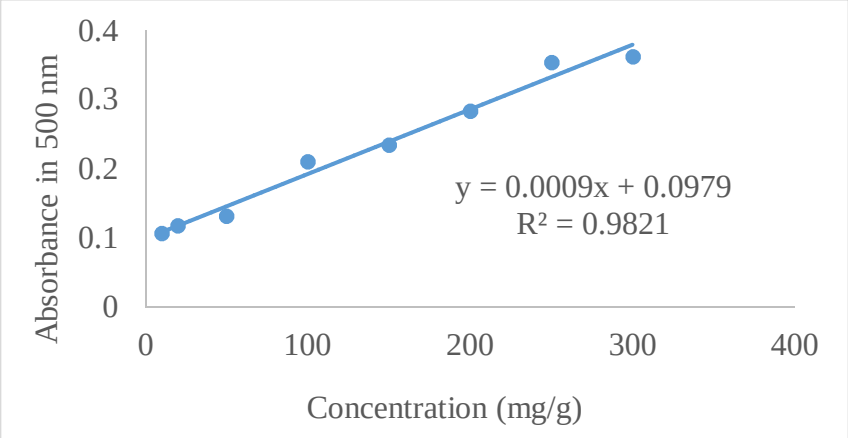

Fig. VII.1: Standard curve of catechin for determination of tannin.

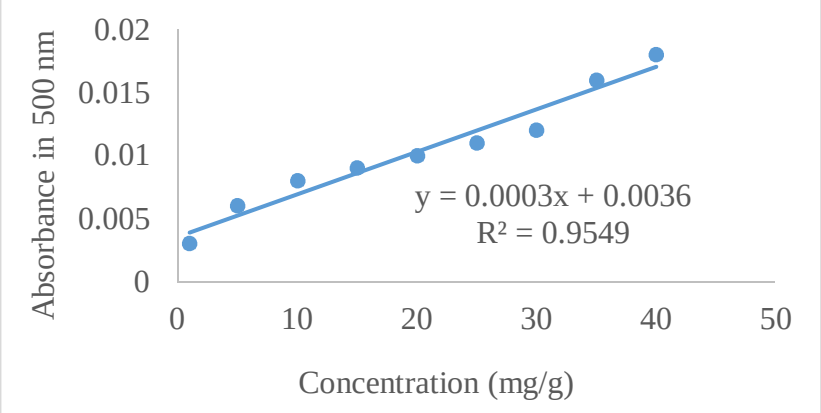

Fig. VII.2: Standard curve of phytic acid for determination of phytate.

Table VII.1: Anti-nutritional parameters of wild fruits in mg/g dried sample

Plants	Oxalate	Tannin	Phytate	Saponin	Alkaloid
G. sapida	6.83 ± 0.34^{a}	0.35 ± 0.03^{a}	4.73 ± 0.01^{a}	0.09 ± 0.02^{a}	0.69 ± 0.07^{a}
E. operculata	4.15 ± 0.38^{b}	0.18 ± 0.03^{a}	5.31 ± 0.02^{b}	0.06 ± 0.03^{a}	0.56 ± 0.02^{a}
A. dioica	6.25 ± 0.96^{a}	0.91 ± 0.03^{b}	7.15 ± 0.01^{c}	0.13 ± 0.02^{b}	1.34 ± 0.02^{b}
A. bunius	3.15 ± 0.45^{c}	$0.68\pm0.02^{a,b,c}$	3.79 ± 0.02^{d}	0.07 ± 0.02^{a}	0.31 ± 0.06^{a}
O. alismoides	8.93 ± 0.93^{d}	$1.03\pm0.01^{b,c}$	5.53 ± 0.01^{b}	0.17 ± 0.03^{b}	1.68 ± 0.02^{b}

Values were expressed as mean of three replicates ± standard deviation; The data with different letters in a column are significantly different from each other at $p < 0.05$.

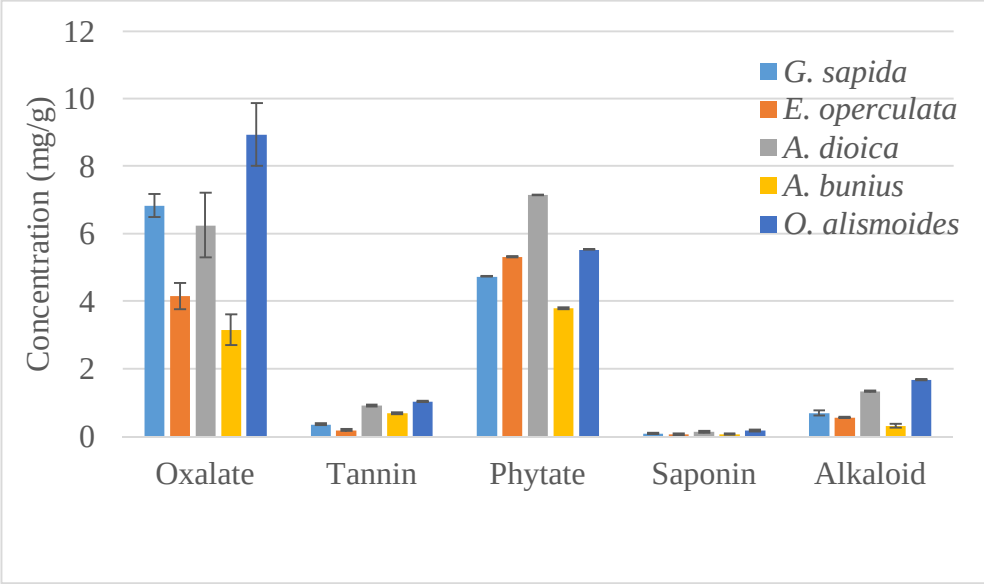

Fig. VII.3: Variation of anti-nutritional contents in mg/g of dried sample.

The level of phytate in the fruits was found the highest in *A. dioica* (7.15 ± 0.01 mg/g) followed by *O. alismoides* (5.53 ± 0.01 mg/g), *E. operculata* (5.31 ± 0.02 mg/g), *G. sapida* (4.73 ± 0.01 mg/g) and *A. bunius* (3.79 ± 0.02 mg/g) and these results are comparable to that of 16 wild fruits reported by Rout and Basak [19]. Mahadkar *et al*. [25] reported phytic acid content in wild edible fruits of *Gmelina arborea* (0.02 g/100 g) and *Oroxylum indicum* (0.04 g/100 g) which was found lower in comparison to the results of the fruits studied herein. The phytate contents of these five wild fruits are found higher than the level of phytate contents in Thai fruits commonly consumed by diabetic patients such as pineapple (0.90 mg/g), mango (0.86 mg/g), guava (0.8 mg/g), durian (0.51 mg/g), dragon (0.39 mg/g) and longan (0.37 mg/g) [26]. Phytic acid is the major storage form of phosphorus in plant tissues. The average daily intake of phytate for humans on vegetarian diets is 2000–2600 mg while it is around 150–1400 mg for inhabitants of rural areas in developing countries on mixed diets [27]. The phytate in food can strongly bind with some essential mineral nutrients in the digestive tract and can result in mineral ion deficiencies [10].

Oxalate content was found the highest in *O. alismoides* (8.93 ± 0.93 mg/g) and the lowest in *A. bunius* (3.15 ± 0.45 mg/g) which is comparable to the oxalate contents of wild edible fruits of Odisha, India reported by Rout and Basak [19]. The higher level of oxalate content was reported in *Ziziphus rugosa* (2.5%) by Rathod and Valvi [28] and in *Ziziphus spichristi* (16.20%) reported by Umaru *et al*. [22]. Oxalate is a concern because it may have

negative effects on mineral availability. Diet rich in oxalates can increase the risk of renal calcium absorption and has been implicated as a major source of kidney stones formation [29].

The concentration of saponin in the fruits ranged from 0.06 ± 0.03 mg/g in *E. operculata* to 0.17 ± 0.03 mg/g in *O. alismoides*. Hess *et al*. [30] reported high saponin contents in tropical fruits such as *Sapindus saponaria* (120 mg/g), *Enterolobium cyclocarpum* (19 mg/g) and *Pithecellobium saman* (17 mg/g) which are higher than the values obtained in the wild fruits of the present study. The saponin content of current study is low compared to 17.80 mg/100 g reported by Gernah *et al.* [31] in the African locust bean fruit pulp. High level of saponin >10% could cause gastroenteritis, manifested by diarrhoea and dysentery [32]. However, it was reported that plant saponin reduces body cholesterol by inhibiting its re-absorption and suppressing protozoa in the rumen by reacting with cholesterol in the protozoan cell membrane [22].

Alkaloid content was found the highest in *O. alismoides* (1.68 ± 0.02 mg/g) followed by *A. dioica* (1.34 ± 0.02 mg/g), *G. sapida* (0.69 ± 0.07 mg/g), *E. operculata* (0.56 ± 0.02 mg/g) and *A. bunius* (0.31 ± 0.06 mg/g) which are lower than the values of alkaloid obtained in *Ficus asperifolia* (6.40 ± 0.11 g/100 g) and *Ficus sycomorus* (5.64 ± 0.41 g/100 g) reported by Nkafamiya *et al*. [33]. Sango *et al*. [34] reported high concentration of alkaloids in *Cleome gynandra* (3.44%) and *Solanum nigrum* (15.160%) and these values are much higher compared to the present study. Anhwange *et al*. [35] also reported alkaloid contents of some indigenous wild fruits which ranged from 0.0097 mg/100 g (*Persea americana*) to 0.1075 mg/100 g (*Diallium guineense*). These results are much lower in comparison to the values obtained in the five wild fruits of the present study. Alkaloids are a group of nitrogenous chemical compounds of plant origin which are toxic to many living organisms. They are known to have pharmacological and anti-physiological effects on human and animals. Alkaloids present in plants may prevent chordate and insects from eating it. Some plant alkaloids have also been reported to cause infertility. Alkaloids cause neurological disorders and gastro-intestinal upsets especially when taken in excess [36].

VII.3 Conclusion

In this study, the fruit of *O. alismoides* exhibited higher levels of anti-nutritional factors such as oxalate, tannin, saponin and alkaloid. The lower levels of oxalate, phytate and alkaloid contents were observed in the fruit of *A. bunius*. The presence of anti-nutritional factors such as oxalate, phytate, tannin, saponin and alkaloid in wild fruits may affect micro-nutrients

absorption and thus make the nutrients unavailable. All the five wild edible fruits contained anti-nutritional factors at varied concentrations and very high levels of anti-nutritional compounds were not observed. Hence, consumption of these fruits may be encouraged. Consumption of raw fruits with high anti-nutritional factors should be discouraged. It is therefore recommended to remove or reduce the levels of anti-nutrients subjecting to different processing methods like cooking, blanching, fermentation, roasting before consumption. Future research is also required to explore different processing technique that will reduce the concentration and effect of anti-nutritional factors in fruits and enhance their nutritive value.

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