CHAPTER VII

Determination of Anti-nutritional Contents of Wild Edible Plants

In addition to minerals and vitamins, plants also contain some compounds which reduce the nutritional quality in plants and these are known as anti-nutritional factors or compounds. These compounds disturb the digestion and absorption of nutrients [1]. These compounds cause harmful effects to the human health in high concentrations [2], but also have beneficial roles if consumed in appropriate amounts [3]. They can help in the prevention of diseases like coronary diseases and cancers [4]. These are synthesized in plants by the normal metabolism which inactivates the availability of some nutrients by chelating the proteins, vitamins and minerals and as a whole the plant become less nutrients [5]. There are numbers of anti-nutritional compounds such as lectins, saponins, protease inhibitors, non-protein amino acids, oxalate, phytate, non-starch polysaccharides, tannins, alkaloids and many others [6]. As some of these compounds are heat sensitive, they can be removed by boiling in water, drying, and roasting. Some of these are easily soluble in water and therefore, can be removed by soaking and washing with water, fermenting in certain p^H and other traditional cooking methods [7, 8].

In this study, the anti-nutritional factors in seventeen wild edible plants from Assam of North East India were investigated and reported.

VII.1 Material and Methods

VII.1.1 Chemicals

The chemicals like $KMnO_4$, H_2SO_4, HCl, methanol, ethanol, diethyl ether, acetic acid $FeCl_3.6H_2O$, NH_4OH, and sulfosalicylic acid were obtained from Merck, Mumbai, India. Catechin, vanillin and phytic acids were obtained from Sigma Aldrich, Bangalore, India.

VII.1.2 Sample preparation

The powdered samples from dried plant species were prepared as per the procedure mentioned in the **Section II.2.3** (**Page No. 73**).

VII.1.3 Determination of oxalate

It was determined by titration method described by Day and Underwood [9]. The powdered sample (1 g) was taken into 100 mL conical flask in which 75 mL of 3 N H_2SO_4 was added and stirred in a magnetic stirrer for 1 h and it was then filtered using Whatman No.1 filter paper. From the filtrate, 25 mL was taken and titrated while it is hot (80–90^oC) against 0.05 M $KMnO_4$ solution until a faint pink colour persist for 30 sec. Oxalic acid was then calculated from 1 mL of 0.05 M $KMnO_4$ = 2.25 mg anhydrous oxalic acid [10, 11].

VII.1.4 Determination of tannin

Quantitative estimation of tannin as catechin equivalent was carried out using vanillin–HCl method [12]. Briefly, 1 g of powder sample was extracted with 10 mL methanol for 24 h and it was vortexed, filtered with Whatman No. 1 filter paper and diluted to 25 mL. After that, 1 mL of extract was taken and 5 mL of reagent mixture (1:1 of 4% vanillin in methanol and 8% concentrated HCl in methanol) was added and mixed thoroughly. After 20 min, the absorbance was taken in 500 nm using UV-Visible Spectrophotometer (Perkin Elmer, Lambda 35, USA). For standard graph preparation, different concentrations of catechin (10–300 µg/mL) were taken and the same volume of the reagent mixture was added and the absorbance was taken against the blank reagent.

VII.1.5 Determination of phytate

The phytate was determined according to the method described by Vaintraub and Lapteva [13]. Briefly, 5 g of powder sample was extracted with 100 mL of 2.4% HCl for 1 h at room temperature and centrifuged at 3000 rpm for 30 min. The clear supernatant was collected for the phytate estimation. To 3 mL of the sample solution, 1mL of Wade reagent (0.03% solution of $FeC1_3.6H_2O$ containing 0.3% sulfosalicylic acid in water) was added and the mixture was vortexed and centrifuged at 4000 rpm for 10 min. The supernatant was collected and absorbance was measured at 500 nm using a UV-Visible spectrophotometer (Perkin Elmer, Lambda 35, 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 samples or standard. The phytates were calculated from the phytic acid standard curve and the results were expressed in mg/g dry weight. For standard graph, a series of solutions 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 vortexed for 5 sec. The mixture was centrifuged at 4000 rpm for 10 min, the supernatant was collected and the absorbance was measured at 500 nm by using distilled water as blank.

VII.1.6 Determination of saponins

Saponin contents were determined by gravimetric method described by [14]. Briefly, 10 g of each powder sample was extracted in 100 mL of 20% ethanol and placed in hot water bath at 55°C for 4 h with continuous stirring and it was filtered with Whatman No. 1 filter paper. This 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 was completely dried in hot air oven for constant weight. After drying in hot air oven, it was then cooled in desiccator and saponin content was calculated by the following formula.

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

Where, W_1 = Initial weight of container (g).

W_2= Final weight of container (g).

VII.1.7 Determination of alkaloid

The alkaloid contents were determined following the method described by Griffiths [15]. Briefly, to 5 g of powdered 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 and washed twice with 1% NH_4OH solution. The precipitates were then dried at 60^oC, cooled into desiccator and then re-weighed until the constant weight was obtained. Alkaloid content was then calculated by the following formula.

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

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 *t*-test at $p < 0.05$.

VII.2 Results and Discussion

The results of the anti-nutritional factors studied in the seventeen wild edible vegetables are given in the **Table VII.1.** The standard graphs for determination of tannin and phytate are shown in the **Figures VII.1** and **VII.2**, respectively. The concentrations of tannin and phytate were calculated from the respective equations (Y = 0.0009x + 0.0979, R^2 = 0.9821) and (Y = 0.0003x + 0.0036, R^2 = 0.9549) obtained from the respective standard graphs and the results were expressed in mg/g dry weight (DW) of sample. The anti-nutritional contents in the wild edible plants studied are presented in **Table VII.1**.

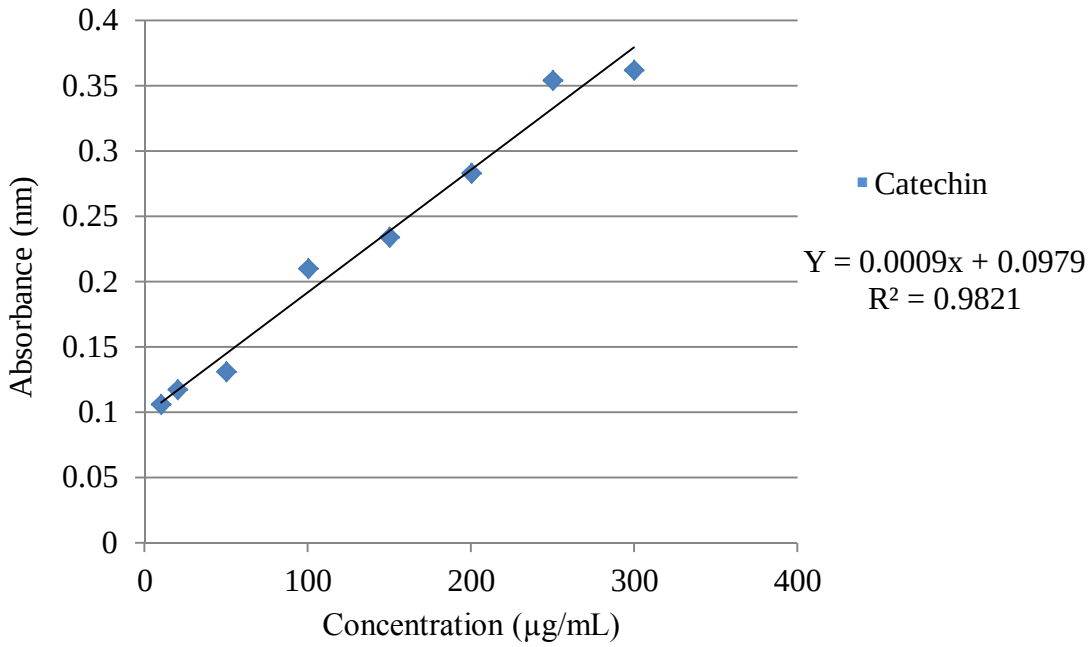

Fig.VII.1: Standard graph for determination of tannin.

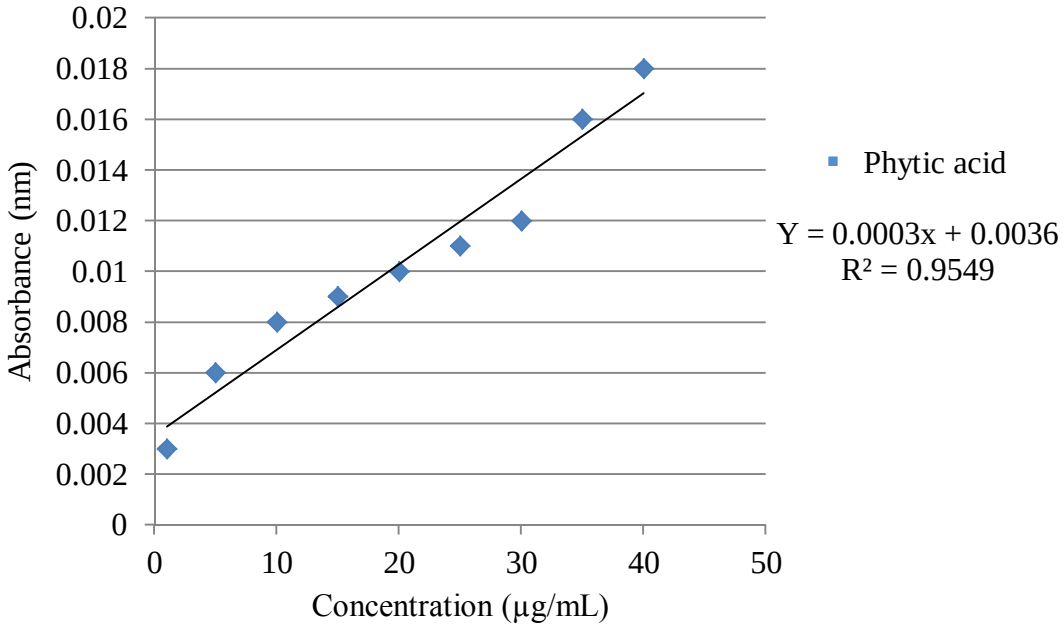

Fig.VII.2: Standard graph for determination of phytate.

From the **Table VII.1**, it is observed that the oxalate content varied from 4.15 ± 0.39 mg/g DW in *S. media* to 39.96 ± 0.23 mg/g DW in *D. cordata*. Higher amounts of oxalate were also found in *C. sinensis* (15.04 ± 0.18 mg/g), *C. hirsuta* (13.65 ± 0.34 mg/g) and *T. angustifolium* (13.12 ± 0.15 mg/g DW). Comparable results of oxalate were reported in some wild fruits [16], and tropical wild vegetables [17]. Singh *et al.* [18] also reported similar oxalate contents in *E. fluctuans* and *E. foetidum* compared to our study. The study of anti-nutritional factors in leafy vegetables from Nigeria reported by Agbaire [19] showed oxalate contents that varied from 0.80 µg/g DW to 2.60 µg/g DW of the sample which are lower in comparison to this study. Similar study was carried on 16 different wild edible fruits by Rout *et al.* [16**]** and reported the highest oxalate content in *Aegle marmelos* as 25.06 ± 6.11 mg/g which is within the range of the results reported in the current study. However, in the previous study reported by Choudhury *et al.* [20], the oxalate contents found in *E. fluctuans, E. foetidum, P. chinensis, S. media* were 0.7 mg/g, 0.6 mg/g, 0.3 mg/g and 0.3 mg/g, respectively which is lower in comparison to the results of present study. In the body, a high concentration of oxalate forms insoluble calcium oxalate complex which hinders the absorption of soluble calcium ions and as a result kidney stone is formed [21]. The oxalate due to its water solubility, it can be removed by washing, boiling, and soaking methods [22].

According to the Oxalosis and Hyperoxaluria Foundation [23], less than 80 mg of oxalate/day is considered as a low oxalate food and therefore, our findings can be considered as low oxalate vegetables.

Table VII.1: Anti-nutritional contents of wild edible plants in mg/g dry weight (DW)

Plants	Oxalate	Tannin	Phytate	Saponin	Alkaloids
Sz	11.02±0.22^{a}	2.74±0.08^{a}	5.69±0.03^{a}	11.00±0.50^{a}	1.68±0.02^{a}
Ch	13.65±0.34^{b}	1.02±0.01^{b}	9.22±0.10^{b}	13.90±0.91^{b}	1.34±0.00^{a}
Nh	8.85±0.12^{c}	2.96±0.01^{a}	5.56±0.05^{a}	10.66±1.06^{c}	0.66±0.02^{b}
Bl	6.735±0.18^{d}	3.21±0.13^{c}	3.87±0.04^{c}	4.86±1.19^{d}	0.13± 0.22^{b}
Sp	9.25±0.36^{e}	0.83±0.11^{b}	8.59±0.01^{d}	4.63±1.20^{d}	0.19±0.01^{b}
Ta	13.12±0.15^{b}	4.32±0.04^{d}	4.76±0.07^{e}	9.30±0.07^{e}	0.65±0.01^{b}
Oj	8.685±0.33^{c}	3.92±0.05^{d}	4.40±0.04^{e}	3.82±1.01^{f}	2.23±0.01^{c}
Mp	6.315±0.92^{d}	4.47±0.01^{d}	7.68±0.01^{f}	14.40±0.30^{g}	3.22±0.01^{d}
Dc	39.96±0.23^{f}	1.16±0.01^{b}	4.11±0.12^{e}	12.60±0.62^{h}	1.30±0.05^{a}
Cs	15.04±0.18^{g}	8.80±0.05^{e}	5.41±0.19^{a}	5.56±1.17^{i}	0.31±0.01^{b}
Sm	4.15±0.39^{h}	0.68±0.02^{b}	3.54±0.04^{c}	12.36±0.41^{j}	1.96±0.01^{e}
Pc	11.68±0.24^{i}	4.10±0.06^{d}	7.50±0.07^{f}	5.30±0.62^{i}	0.69±0.01^{b}
Aa	6.82±0.34^{d}	12.96±0.04^{f}	4.15±0.01^{e}	13.30±0.40^{k}	1.10±0.02^{a}
Efo	6.87±0.30^{d}	1.00±0.05^{b}	3.85±0.07^{c}	1.43±0.20^{l}	0.56±0.01^{b}
Lj	10.39±0.29^{j}	2.44±0.07^{a}	7.20±0.08^{f}	3.23±0.30^{m}	1.28±0.01^{a}
Pp	9.49±0.47^{e}	1.30±0.13^{b}	4.60±0.17^{e}	14.13±0.92^{g}	0.89±0.01^{b}
Ef	6.07± 0.13^{k}	1.96±0.08^{g}	3.84±0.03^{c}	11.66±2.13^{n}	1.69±0.01^{a,e}

Sz = S. zeylanica, Ch = C. hirsuta, Nh = N. herpeticum, Bl = B. lanceolaria, Sp = S. peguensis, Ta = T. angustifolium, Oj = O. javanica, Mp = M. perpusilla, Dc = D. cordata, Cs = C. sinensis, Sm = S. media, Pc = P. chinensis, Aa = A. acidum, Efo = E. foetidum, Lj = L. javanica, Pp = P. perfoliatum and Ef = E. fluctuans, DW = Dry weight, Values were expressed as mean of three replicates ± standard deviation and the data with different letters in a column are significantly different from each other at $p < 0.05$.

The tannin content in the plant species was found to be ranging from 0.68 ± 0.02 mg catechin equivalent (CE)/g DW in *S. media* (lowest) to 12.96 ± 0.04 mg CE/g DW in *A. acidum* (highest). The higher levels of tannin were also detected in *C. sinensis, M. perpusilla, T. angustifolium, P. chinensis* and *B. lanceolaria* (**Table VII.1**). Similar

results of tannin were also reported by Suneja *et al*. [24] in black gram seeds. However, Agbaire [19] reported low amounts of tannin that ranged from 0.04 ± 0.01 μg/g to 0.26 ± 0.03 μg/g in some leafy vegetables of Nigeria. However, in previous study reported by Choudhury *et al*. [20], the tannin contents in *E. fluctuans, E. foetidum S. media* and in *P. chinensis* were 6.6 ± 1.13 mg/g, 14.5 ± 1.43 mg/g, 3.7 ± 0.55 mg/g and 13.3 ± 1.03 mg/g, respectively, where some results are comparable to the results of current study. Similarly, Singh *et al.* [18] reported higher levels of tannin in *E. fluctuans* and *E. foetidum* in comparison to this study. Tannins have an astringent response in the mouth, hamper dietary iron absorption and can cause a growth depression [25].

In this study, the concentration of phytate was found the lowest in *S. media* (3.54 ± 0.04 mg/g DW) and it was found the highest in *C. hirsuta* (9.22 ± 0.10 mg/g DW). Similarly, close values of phytate were reported in some leafy vegetables of Ghana [26], some food and tropical vegetables of Nigeria [27, 17] and some wild fruits of Odisha, India [16]. However, Agbaire [19] reported lower levels of phytate content in some leafy vegetables [28] and underutilised leafy vegetables [29]. The phytate contents found in the current study were slightly higher in comparison to the previous study [20, 18] reported on *E. fluctuans, E. foetidum, P. chinensis, S. media* which were 2.7 ± 1.11 mg/g, 1.8 ± 0.34 mg/g, 0.6 ± 0.01 mg/g and 0.3 ± 0.01 mg/g, respectively. It has been reported that phytic acid can bind with several important minerals like zinc, iron, phosphorus, calcium, etc. and they form insoluble phytate complexes which are not being able to be absorbed by the body and thereby reduce their bioavailability and digestion in humans [30].

The saponin contents in the selected wild vegetables ranged from 1.43 ± 0.02 mg/g DW which is the lowest value in *E. foetidum* to 14.40 ± 0.03 mg/g DW which is the highest value in *M. perpusilla*. The higher levels of saponin were also detected in *P. perfoliatum, C. hirsuta, S. media, A. acidum, D. cordata* and *E. fluctuans* (**Table VII.1**). Our results are comparable to the findings of some reported studies on non-conventional vegetables [26], traditional vegetables [18], wild edible fruits [29] and black grams [24]. However, the saponin contents of the present study were higher in comparison to the saponin contents of *E. fluctuans* (4.1 ± 0.56 mg/g) and *S. media* (3.8 ± 0.04 mg/g) reported by Choudhury *et al*. [20]. On the other hand, Agbaire [19] reported lower levels of saponin contents in some leafy vegetables such as in *Amaranthus flavus, Corchorous olitorius,* and *Solanum monocarnum* and Pramodini *et al*. [16] reported higher saponin contents in some wild fruits. Due to complex nature of structures of saponins present in plants, they impart several chemical and biological properties such

as bitterness, foaming and emulsifying, haemolytic, insecticidal and antimicrobial properties [31].

In this study, the alkaloid contents were found to be ranging from 0.13 ± 0.22 mg/g DW in *B. lanceolaria* to 3.22 ± 0.01 mg/g DW in *M. perpusilla* (highest). These findings are similar to the alkaloid contents of some leafy vegetable [26], edible flowers [32], and fruit berry [33]. However, Nkafamiya *et al.* [34] reported higher levels of alkaloids in *Ficus asperifolia* (6.40 ± 0.11 g/100 g) and *Ficus sycomorus* (5.64 ± 0.41 g/100 g). Sango *et al.* [35] also reported higher concentration of alkaloids in *Solanum nigrum* (15.16%) compared to the present study. On the other hand, Anhwange *et al.* [36] reported very low alkaloid contents in some 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 results obtained in the 17 wild plants of present study. Alkaloids are organic molecules containing nitrogen of plant origin which are toxic to many living organisms. They have anti-physiological and pharmacological effects on human and animals. Alkaloids present in plants can prevent insects from eating it. Some plant alkaloids have also been reported to cause infertility. Alkaloids when consumed in excess cause gastro-intestinal upset and neurological disorders [37]. However, most of the anti-nutritional factors are removed during the cooking processes and thus it is expected not to find any or only minute concentration of anti-nutritional compounds which will not produce any lethal effect to the prospective consumers [32].

VII.3 Conclusion

In this study, anti-nutritional contents such as oxalate, tannin, phytate, saponin and alkaloids were evaluated and variable amounts of anti-nutritional contents were observed which may be due to different plant species, locations and environmental conditions. Very high levels of anti-nutritional contents were not found in the current study except in some plant species such as *D. cordata* which exhibited the highest level of oxalate content (39.96 ± 0.23 mg/g DW) and *M. perpusilla* which showed the highest saponin content (14.40 ± 0.30 mg/g DW). However, presence of anti-nutritional compounds also shows several medicinal properties including antioxidant and antimicrobial properties.

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