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CHEMICAL ANALYSIS AND NUTRITIONAL AMOUNT OF *TRICHOSANTHES BRACTEATA* (LAMK.) VOIGT. AND *COCCINIA INDICA* W&A

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Nutritional therapy and phytotherapy have emerged as new concepts and healing systems have quickly and widely spread in recent years. Strong recommendations for consumption of nutraceuticals, natural plant foods, and the use of nutritional therapy and phytotherapy have become progressively popular to improve health, and to prevent and treat diseases. With these trends, improving the dietary nutritional values of fruits, vegetables and other crops or even bioactive components in folk herbals has become targets of the blooming plant biotechnology industry. The present study aims to evaluate the nutrients and antinutrients of two wild climbing cucurbitaceous fruit plants such as *Coccinia indica* and *Trichosanthes bracteata*. Nutrition and health care strongly interconnected and many plants have been consumed both as food and for medicinal purposes. The consumption of non – cultivated botanicals play a central role in the diet, but very few ethnopharmacological and phytopharmacological studies have dealt exhaustively with the potential health benefits of such diets. Reverse phase high performance liquid chromatography and analytical were the methodologies adapted for the whole study. *Coccinia* and *Trichosanthes* species had significantly high phenolic content (0.5mg / g fr.wt. to 0.7 mg / g fr.wt.). A positive and strong correlation was observed between phenols and phenolic acids such as cinnamate, coumarate, caffeate, chlorogenate, ferulate, gallic and hydroxyl benzoic acids among the taxa. Trypsin protease inhibition and α- amylase inhibition was found to be significant in *Trichosanthes* than *Coccinia* . As the trypsin inhibitor and amylase inhibition contents are higher in *Trichosanthes* than in *Coccinia* it is tempting to suggest that these compounds, together with other yet- unknown deleterious substances, may have led to the overall toxicity in the plant.

Keywords: Antinutrients; Amylase inhibition; Nutraceuticals; Phenolic acids; Phytonutrients; Trypsin protease inhibition; Wild plants.

Introduction

Nearly 60% of the world's food supply comes from rice, wheat and corn[1] although approximately 250,000 plant species have been described worldwide[2]. Wild edible plants contribute significantly to the nutrition of rural inhabitants. Although these foods are consumed by people throughout the year in fresh and dried forms, reliance on these foods increases during periods of cereal shortages. Nowadays, however, a nutritional transition is occurring in the poorest countries of the world resulting in the replacement of traditional plant-based diets that are rich in fruits and vegetables with diets that are rich in calories provided by animal fats and sugars, and low in complex carbohydrates Research on wild fruits and other wild edible plants is also intended to promote the preservation of these species, presently under threat by human activities. In addition to their nutritional value, the preservation of these fruits also has economical advantages, as there is a significant trade in some if these wild edible fruits. Some of these wild fruits are also known to have medicinal properties. Any scientific evidence for the health benefits of such wild fruits in addition to their nutritional value would be an added value to the plants producing such fruits. Concerning their medicinal properties, the most commonly studied benefit is their antioxidant effects. Antioxidants play a crucial role in the prevention of chronic ailments such as heart disease, cancer, diabetes, hypertension, stroke and Alzheimer's disease by combating oxidative stress. The dietary intake of fruits has a strong inverse correlation with the risk of developing coronary heart disease and cancer. In fruits, vitamins C, A and E, and polyphenols are known to be responsible for such antioxidant activity, with polyphenols being the most active. A number of studies have reported the content of some essential nutrients in wild edible fruits from India, including energy levels, ascorbic acid, vitamins, metals and trace minerals.

Seeds of many plants even though they are rich in protein, contain a large number of antinutritional substances, which hinder free nutritional utilization in monogastric animals and humans. Plant proteinase inhibitors (PIs) have been well established to play a potent defensive role against predators and pathogens. Although diverse endogenous functions for these proteins has been proposed, ranging from regulators of endogenous proteinases to act as storage proteins, evidence for many of these roles is partial, or confined to isolated examples.

Coccinia indica a climbing perennial herb with ovoid fruits and are used as vegetable when green, and eaten fresh when ripe. *Trichosanthes brateata* is a large perennial dioecious climber with globose fruits. The major objective of the present study was to investigate nutraceutical and antioxidant components in the fruits of these two wild cucurbitaceous members.

Material and Methods

Plant materials: The plants *Coccinia indica* and *Trichosanthes brateata* were collected growing wild along the foot hills of Agasthimala. The plants are consumed by the local people as vegetables. Fresh leaves and fruits were used for the whole study.

Estimation of total carbohydrates: Total carbohydrates present in the samples were extracted using methanol and estimated as per the methodology of Dubois *et al*[3]. The absorbance was measured at 490nm.

Total protein: The total protein was extracted with phosphate buffer pH 7.0 and estimated following the method of Bradford *et al*[4].

Antinutritional factor analysis: Tannins was quantified spectrophotometrically using Folin- Dennis reagent[5]. Extraction was done with methanol / water. Tannic acid was used as standard. Phytic acid content was determined by the method of Ravindran and Ravindran[6].

Isolation and assay of trypsin protease inhibitor and amylase protease inhibitor : 3g of the plant tissue was homogenized with 15ml distilled water Incubate the filtrate for 2 to 3hrs in a refrigerator with occasional stirring. Centrifuge the filtrate at 12000 rpm for 20min at 4°C. The supernatant was used as the inhibitor extract. TPi was quantified as per the method of Kakade *et al*[7].

Estimation of α-amylase inhibitors (AI): α-Amylase inhibitors were extracted from the tissues using sodium phosphate buffer (0.02 M; pH 6.9) containing 0.3 M NaCl. The AI was quantified in the extracts by the method described by Rekha *et al*[8].

Estimation of total phenols and phenolic acids: Total phenolic content was extracted by distillation with 80% methanol by the method of Mayer *et al.*[9]. Fractionation of phenolic acids in the fruit samples was carried by RP-HPLC following the method of Beta *et al.*[10] using internal standards.

Result and Discussion

Proximate composition: The proximate analysis of leaves, fruits and seeds of *Coccinia indica* and *Trichosanthes bracteata* are presented in Table 1. The moisture content of the fruits ranges from 91% to 93%, total ash (4.7 – 7.2 %), crude fibre (1.6 - 3%), respectively. The results of the proximal composition of fruits indicate appreciable variation in crude protein content and total sugar. The crude protein in the fruits of *C. indica* was higher (13.5 mg/g) than that of *T. brateata* (11.2 mg / g). The seed protein value was more or less similar in both the species i.e., 30.8 and 32.8 mg/g, respectively, a value within the range found for cereal seeds (84.0 to 148 g Kgy^{-1} dry matter) such as corn, triticale and wheat[11]. The data for seed protein of the two species were significant than the protein contents in seeds of edible legumes (180±250 g Kgy^{-1} dry matter)[12]. The values are also comparable to the seed protein contents of underutilized legumes such as *Canavalia ensiformis*, 260 g Kgy^{-1} dry matter[13], *Bauhinia purpurea*, 271.7 g Kgy^{-1} dry matter[14] and some species of *Crotalaria* (200.0±396.0 g Kgy^{-1} dry matter)[15]. The leaf protein content was also similar in both the species. Total fruit carbohydrates in *C. indica* (107.8 mg/g) was two fold higher than that of *T. brateata* (54.1 mg/g), whereas the seed carbohydrates shows a reverse trend i.e., higher in *T. brateata* (73.7 mg/g) than in *C. indica* (14.3 mg/g). Leaf carbohydrate content does not show much variation among the species (Table 1).

Antinutritional compounds: Figure 1 a and b represent the results of antinutritive constituents such as total free phenolics and tannins in fruits, leaves and seeds. The presence of antinutritional factors adversely affects the nutritional qualities of many wild and cultivated fruits. A preliminary evaluation of some of these factors was made in *Coccinia* and *Trichosanthes.* Phytic acid content was not observed in the leaves, fruits and seeds of both the species. The tannin content in the fruits of both the species was more or less similar i.e., 1μg/g tissue where as in leaves and seeds the tannin content show variation i.e., seed tannin content in *Trichosanthes* was 4.4 μg/g but it was comparatively low in *Coccinia* (3 μg/g). The total phenolic content was 0.5 and 0.7 mg / g in the fruits of *Coccinia* and *Trichosanthes* respectively. The same is with the case

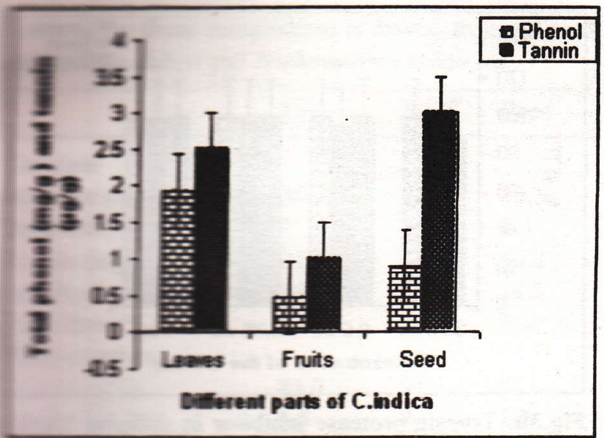

Fig.1a. Total phenol and tannin content in different parts of *Coccinia indica*.

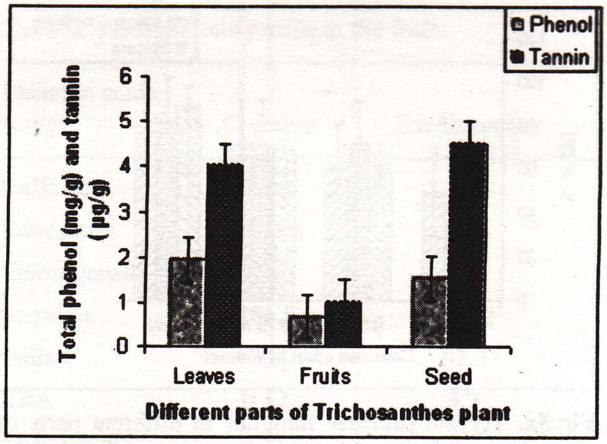

Fig.1b. Total phenol and tannin content in different parts of *Trichosanthes bracteata*.

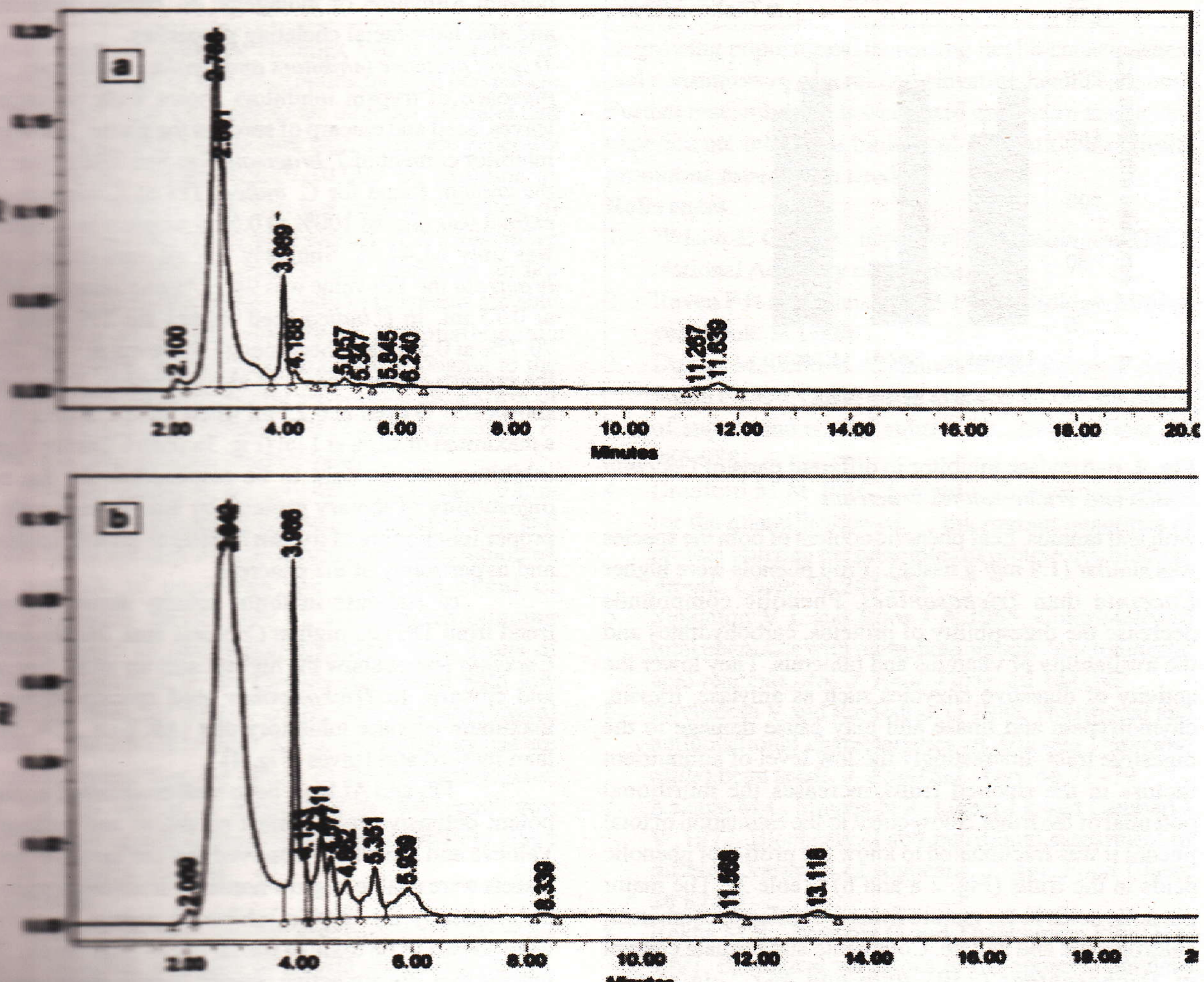

Fig.2a&b. RP-HPLC chromatogram of mature fruits of *Trichosanthes* and *Coccinia*.

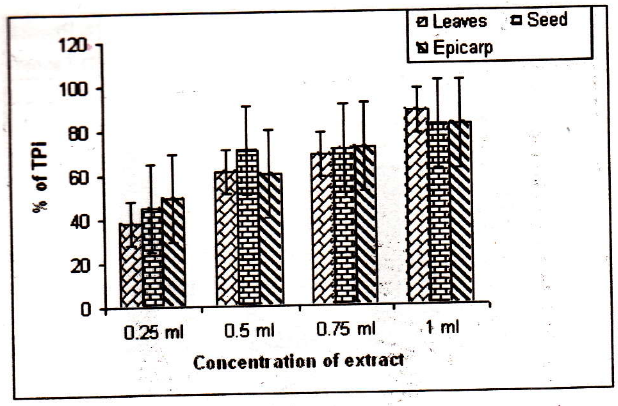

Fig.3a. Trypsin protease inhibitor in different parts of *Coccinia indica.*

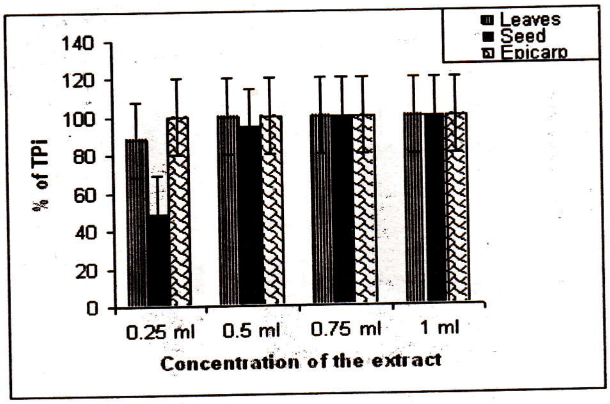

Fig.3b. Trypsin protease inhibitor in different parts of *Trichosanthes bracteata.*

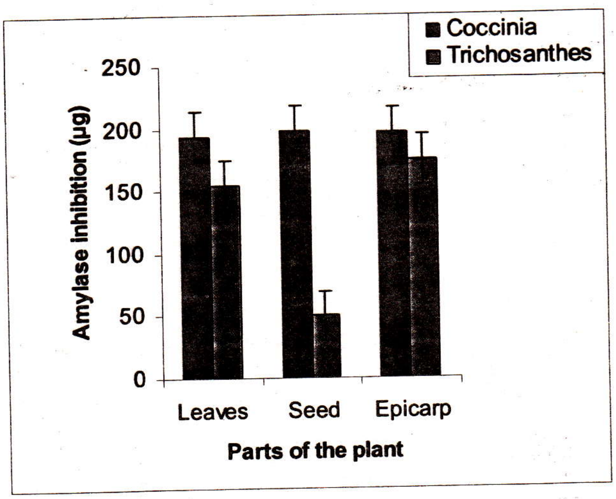

Fig. 4. α-Amylase inhibitor in different parts of *Coccinia indica* and *Trichosanthes bracteata.*

with leaf tannins. Leaf phenolic content of both the species was similar (1.9 mg/ g tissue). Fruit phenols were higher *Coccinia* than *Trichosanthes*. Phenolic compounds decrease the digestibility of proteins, carbohydrates and the availability of vitamins and minerals. They lower the activity of digestive enzymes such as amylase, trypsin, chymotrypsin and lipase and may cause damage to the digestive tract. Interestingly the low level of antinutrient factors in the ripened fruits increases the nutritional potential of the fruits. Subsequent to the estimation of total phenol it was fractionated to know the profile of phenolic acids in the fruits (Fig. 2 a and b) (Table 2). The major phenolic acids in the mature fruits are caffeate, coumarate, chlorogenate and gallate. Coumarate and ferulate content in *Trichosanthes* (2301 µg/g and 462.2 µg/g) was significant than in *Coccinia* (1434 µg/g and 287 µg/ g). These phenolic acids are effective antioxidants because they scavenge reactive oxygen species, trap nitrate and prevent formation of mutagenic N- nitroso compounds and also have metal chelating properties.

Trypsin protease inhibitors and amylase inhibitors: The presence of trypsin inhibitors shows wide variation in leaves, seed and epicarp of seeds in the plants. The trypsin inhibitor content of *T. bracteata* was two fold higher than the content found for *C. indica*. TPi of *T.bracteata* leaf extract was almost 100% at 0.5 ml whereas in *C.indica* it was only 61.44 %. Similarly 0.5 ml seed extract of *T. bracteata* the TPi value was 94.71 % and becomes 100 % at 0.75 ml. In *C.indica* seed extract the TPi value was 70.7 % at 0.5 ml and becomes 81.9% even at 1 ml. TPi in the epicarp of the seeds was also analysed. *T. bracteata* shows 100 % even at 0.25 ml where as in *C.indica* it was a maximum of 82 % at 1 ml (Fig. 3a and b). Dietary trypsin inhibitors are thought to be responsible for the poor digestibility of dietary protein, by interference with the proper functioning of trypsin leading to growth inhibition and hypertrophy of the pancreas.

α- Amylase inhibitor activity shows a reverse trend from TPi i.e., high in *Coccinia* than *Trichosanthes*. *Coccinia* leaves show the highest activity of AI than seed and epicarp. In *Trichosanthes* seed epicarp shows the maximum amylase inhibitory unit (AIU) i.e., 174.5AIU, than its seed and leaves (Fig. 4).

TPi and AI have been well established to play a potent defensive role against predators and pathogens. Valueva and Mosolov[16] observed that the larvae of certain insects were unable to grow normally in soybean products. Subsequently the trypsin inhibitors present in soybean were shown to be toxic to the larvae of flour beetle. This reveals that PIs are active against certain insect species. The PIs interact with their target protease by contact with the active site of the protease resulting in the formation of

Table 1. Proximal composition in leaves, fruit and seed of *Coccinia indica* and *Trichosanthes bracteata*.

	Leaves	Fruit	Seed
Coccinia			
Moisture (%)	86.2	91	75.5
Total ash (%)	2	4.7	0.5
Protein (mg/g)	21.9	13.5	30.8
carbohydrates (mg/g)	38.9	107.8	14.3
Total fibre (%)	2.4	1.6	0.8
Trichosanthes			
Moisture (%)	83.9	93	51
Total ash(%)	3.5	7.2	1.7
Protein (mg/g)	20.4	11.2	32.8
carbohydrates (mg/g)	31.8	54.1	73.7
Total fibre(%)	3.8	3	1

Table 2. Phenolic acid profile in the fruits.

Phenolic acids (µg/g)	*Coccinia*	*Trichosanthes*
Caffeate	2.64	10.5
Coumarate	1433.8	2301
Chlorogenate	52.99	77.8
Ferrulate	286.8	462.2
Gallate	42.4	62.2
HBA	0.12	4.3
Paracatechol	ND	1.5
Vanillate	0.16	0.6
Cinnamate	2.15	77.3

a stable protease inhibitor complex that is incapable of enzymatic activity. In *Coccinia indica* the high percentage residual activity of trypsin protease inhibitor indicates that it may belongs to Bowman-Birk type. BBIs are generally low molecular weight (8000-10,000 D) proteins as compared to Kunitz type. The low residual activities of TPi in the presence of the seed extract of *Trichosanthes bracteata* reveals that Chymotrypsin inhibitors do not occur in the seeds. Proteinase inhibitors in plants are able to suppress enzymatic activity of phytopathogenic microorganisms. Inhibitors from beans belonging to the Bowman.Birk inhibitor family suppressed the growth of hyphae and conidium germination of *Fusarium solani*, *F. culmorum*, and *Botrytis cinerea* fungi[16].

Protease inhibitors are very effective in their ability to suppress carcinogenesis in many different *in vivo* and *in vitro* assay systems. Anticarcinogenic protease inhibitors are capable of reversing the initiating event of carcinogenesis presumably by stopping an ongoing process begun by carcinogen exposure. High levels of protease inhibitors in the diet are associated with low incidence rates for breast, colon, oral and pharyngeal cancers. The effects of most chemo preventive agents are far more limited than the effects of the anti carcinogenic PIs. PIs can almost be considered as "universal" anti carcinogenic agents in their wide ranging ability to affect the carcinogenic process.

Conclusion

The present study indicates that wild cucurbitaceous fruits can be classified as source of protein and carbohydrates. Similarly the high protease inhibitors also reveal its medicinal potentiality. The emergence of such underutilized fruits can be ascribed to multiple factors such as growing populations, increasing health consciousness and consumers in general and elevating health care cost. Further research work is warranted on *in vitro* and *in vivo* experiments related to biological evaluation and health promoting aspects one needed.

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