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Inorganic and Phytochemical Content Analysis of the Wild Abyssinian Thyme Spice

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Abstract

A study was conducted to investigate selected inorganic, anti-nutritional factor and pro-vitamin "A" contents of the aromatic spices of the wild Abyssinian thyme aerial parts. Different aerial parts of the plant were considered for the experiments. The mineral content, anti-nutritional and pro-vitamin"A" value of the plant parts was analyzed using AOAC (2000). Mineral contents such as iron (Fe), zinc (Zn), calcium (Ca), copper (Cu) and magnesium (Mg) were determined by Atomic Absorption Spectrophotometer and Condensed tannin, total phenolic content, carotenoids, beta carotene and retinol equivalent vit "A" were determined by UV/vis Spectrophotometer and the data were analyzed by SAS software version 9.1. The results revealed that Abyssinian thyme herb aerial parts (F, L & W) contained Zinc (9.19, 9.01 and 8.36 mg/100 g); Iron (0.022, 0.0022 and 0.012 mg/100 g), copper (0.038, 0.054 and 0.045 mg/100 g), Magnesium (2.47, 2.32 and 2.1 mg/g) and Calcium (392, 379.5, and 355.3 mg/g), respectively. They contained condensed tannin (0.2, 0.4 and 0.9 µg/mL), total phenolic content (0.5, 0.2 and 0.3 µg/mL) of anti-nutritional factors, respectively. Furthermore, they contained carotenoids (59.6, 68.7 and 63.0 IU), beta carotene (119.5, 137 and 126.7 IU) and retinol equivalent (239.5, 274.8 and 252 IU) of pro-vit "A", respectively. Among the minerals in Abyssinian thyme herb aerial parts calcium is the highest in the flower part and the lowest was in the whole thyme aerial parts, the least of the five minerals is the iron and copper. None of the mineral contents were highest for the whole plant. From the wild Abyssinian thyme aerial parts the flower had highest total phenolic content and the leaf had the lowest and data shows that the whole thyme plant had the highest condensed tannin content from the three aerial parts wild Abyssinian thyme spice.

Keywords: zinc, Thyme, Iron, condensed tannin, Carotenoids

1. INTRODUCTION

The name thyme, in its Greek form, was first given to the plant by the Greek's as a derivative of a word which meant "to fumigate", either because they used it as incense, for its balsamic odor, or because it was taken as a type of all sweet smelling herbs. Others say the name derives from the Greek words, *Thymon*, meaning perfume or famous, signifying courage, the plant being held in ancient and medieval days to be a great source of invigoration, its pleasant qualities inspiring courage.The genus *Thymus L.* belongs to the family *Lamiaceae*, and consists of about 215 to 350 species, according to different literature data (Cronquist, 1988; Zaide, 2005; Crow, 2005). They are usually herbaceous perennials, small shrubs occurring within the Mediterranean region, which is a center of the entire genus, and are also characteristic of Asia, Southern Europe and North Africa (Maksimovic *et al*., 2008).

Thymus schimperi L. (Labiatae), known by the local language name "*Tossign"* is indigenous into northern Ethiopia, which is a small perennial herb shrub of very high altitude areas. The herb which is wild, though not yet cultivated, is a useful condiment. It is also reportedly used in the control of gonorrhea (Demsew, 1993). Furthermore, when it is added to boiling water and drunk, it helps against cough and liver disease (Demsew, 1993). As a spice, *Tossign* is dried, ground and mixed with other spices that are used in the preparation of "*Berber (*ripen, dried and ground red pepper*)*" and "*Shirro (*powder form of roasted, dehulled and ground legume grains*)*". In some provinces, it is used to flavor tea or drunk alone as tea (Demsew, 1993).

Traditionally, from the wild Abyssinian thyme herb aerial parts of the leaf; people in the central and northern highlands are used to make flavored tea and spices condiments and consumed for a long time till now. It has no yet studied the proximate composition and is all traditional preparation and usage in Ethiopia. Emba Alagi *(*mount Alagi) was one of the highland Woreda's of southern Tigray, northern Ethiopia, which is also known for wild thyme herb leaves used for preparation of *Shiro,* Berbere and herbal tea. This study aimed to investigate the proximate composition and mineral analysis of the aerial parts of wild Abyssinian thyme plant.

2. MATERIALS AND METHODS

2.1. Collection of Plant material

Samples of Abyssinian thyme herb were collected in August, (2015) during the flowering period in northern Ethiopia, Tigray southern highland (mount Alagi). The thyme aerial parts were dried under shade of the house over the plastic sheet covered with muslin cloth and then spread over the samples. The dry samples were packed in black polyethylene plastic bags; transported to Haramaya University and stored in dry, dark and cool area in the laboratory until required for laboratory tasks and analysis.

2.2. Grinding
The three separated thyme herb aerial parts were taken and ground to powdered form by using a grinder.

2.3. Analysis of Selected inorganic contents
Mineral analysis was determined according to atomic absorption spectrophotometer (Model 2010 VGP Spectrophotometer, Buck Scientific, East Norwalk, CT, USA) (db)
Principle: In this technique the atoms of an element were vaporized and atomized in the flame. The atoms then absorb the light at a characteristic wavelength. The source of the light is a hollow cathode lamp, which is made up of the same element, which has to be determined. The lamp produces radiation of an appropriate wavelength, which, while passing through the flame is absorbed by the free atoms of the sample. The absorbed energy is measured by a photo-detector read-out system. The amount of energy absorbed is proportional to the concentration of the element in the sample. The digested sample was analyzed for the spectrophotometer.
The concentrations of minerals recorded in terms of "ppm" were converted to milligrams (mg) of the minerals by multiplying the ppm with dilution factor and dividing by 1000, as follows:

$$\text{MW (ppm)} = \frac{\text{Absorbency (ppm) X Dry Wt XD}}{\text{Wt. Sample X1000}}$$

Where: MW= minerals, mass weight, D= dilution factors, wt. =weight
Note: Dilutions for magnesium and calcium were 20xs and 40xs and for other minerals, including zinc, iron, and copper were 100.

2.4. Analysis of selected anti-nutritional factors of thyme herb aerial parts
2.4.1. Total phenolic content
The total polyphenol content (TPC) was determined by spectrophotometer, using Gallic acid as standard, according to the method described by the International Organization for Standardization (ISO 14502-1, 2005). Briefly, 0.1g for powder and 0.1 mL of tea infused of the diluted sample extracts were transferred in duplicate to separate tubes containing 5.0 mL of a 1/10 dilution of Folin -Ciocalteu's reagent in the water. Then, 4.0 mL of a sodium carbonate solution (7.5% w/v) was added. The tubes were then allowed to stand at room temperature for 60 min before absorbance at 765 nm using a spectrophotometer (6505 / uv /vis spectrophotometer, Model 6505, U.K, GENWAY) was measured against water. The TPC was expressed as Gallic acid equivalents (GAE) in g/100 g material. The concentration of polyphenols in the samples was derived from a standard curve of Gallic acid ranging from 10 to 50 µg/mL (Pearson's correlation coefficient: r^2=0.9996).

$$\text{Total phenolic contents(ppm)} = \frac{\mu\frac{\text{g}}{\text{ml}} * Df * 100}{sample\ mass(g\ or\ ml)}$$

Where: µg/ml= absorbency reading concentration
Df= dilution factor

2.4.2. Condensed tannins
The condensed tannin content of the sample was determined according to the modified Vanillin-HCl methanol method as described by (Price *et al.,* 1978). The Vanillin-HCl reagent was prepared by mixing equal volumes of 8% concentrated HCl in methanol and 1% Vanillin in methanol. The solutions of the reagent has been mixed just prior to use. About 0.2 g of the ground sample and 0.2ml of the infused tea extracted were placed in a small conical flask. Then 10 ml of 1% HCl in methanol was added. The conical flask was capped and continuously shaken for 20 minutes and the content, then centrifuged at 400 rpm for 10 minutes. About 1.0 ml of the supernatant was pipetted into a test tube containing 5 ml of Vanillin-HCl reagent. Absorbance at 500 nm was read using spectrophotometer(6505 / UV /vis spectrophotometer, Model 6505, U.K, GENWAY) after 20 minutes incubation at 30°C, a blank sample was carried out with each run of the sample. A standard curve was prepared expressing the result as catechin equivalent, i.e. catechin (mg/ml). Tannin content was expressed as catechin equivalent as follows:

$$\text{Tannin}(\%) = \frac{(\text{C} \times 10) \times 100}{200}$$

Where: C= Concentration of corresponding to the optical density
10 = volumes of the extract (ml), 200 = sample weight.

2.5. Pro-vitamin "A" (crotenoid, retinol equivalent and beta -carotene) contents
All the reagents used were of analytical grade and (6505 UV/vis spectrophotometer, model 6505, U.K, GENWAY) was used for the absorbent measurements. The chlorophylls and carotenoids were extracted with

ethanol (100% alcohol). According to the methods described by (Kukric *et al,*. 2012 and Chang *et al*., 2013). The chlorophylls, and carotenoid content were analyzed spectrophotometrically by absorption measurements (A) at 440, 649 and 665 nm (6505 UV/vis spectrophotometer, Model 6505, U.K, GENWAY) and calculated according to the following equations:

$$\text{Chlorophyll a}\left(\frac{\text{mg}}{\text{g}}\right) = \frac{13.7\text{A}665 - 5.76\text{A}649}{\text{mass}(200)}$$

$$\text{Chlorophyll } b\left(\frac{mg}{g}\right) = \frac{25.8A649 - 7.56A665}{Mass(200)}$$

$$\text{Carotenoids}\left(\frac{\text{mg}}{\text{g}}\right) = \frac{4.7\text{A}440 - 0.263\text{C}(\text{chla} + \text{chlb})}{\text{Mass}(200)}$$

Where A=is absoebency reading microgram per milliliter
Calculation of beta carotene:

$$\beta - \text{carotene} = \frac{\mu\text{g per mL} * \text{dilution factor}}{\text{Sample weight}(\text{g})}$$

In many years application, a system of equivalence, in which an International Unit (IU) was equal to 0.3 µg of retinol equivalent, 0.6 µg of β-carotene, or 1.2 µg of other provitamin-A carotenoids was used (USDA, 2008).

3. RESULTS AND DISCUSSIONS

This study conducted to know the inorganic, anti-nutritional factor and pro-vitamin "A" contents of locally available the wild Abyssinian thyme herb by using standard procedures. The results obtained are discussed in the sections that follow.

3.1. Inorganic Contents of the Wild Abyssinian Thyme Herb Aerial Parts

Using standard procedures, the mineral contents of the Abyssinian thyme aerial part products were determined and presented in Table 1. The data showed that the Abyssinian thyme flower, leaves, and whole aerial parts contained Zinc (9.19, 9.01 and 8.36 mg/100 g, respectively); Iron (0.022, 0.0022 and 0.012 mg/100 g, respectively), copper (0.038, 0.054 and 0.045 mg/100 g, respectively), Magnesium (2.47, 2.32 and 2.1 mg/g respectively) and Calcium (392, 379.5, and 355.3 mg/g, respectively). Among the minerals in Abyssinian thyme herb aerial parts calcium is the highest in the flower part and the lowest was in the whole thyme aerial parts and the amount of magnesium and zinc were the highest and lowest in that order. The least of the five minerals are the iron. It can be observed that statistically significant differences existed among the three parts of the herb in all the five mineral contents. The flower part was observed to have the highest calcium, magnesium, and zinc and the lowest copper and iron contents. The leaves were found to have the highest calcium content of the three and the lowest iron content. None of the mineral contents were highest in the whole plant, indicating that its mineral contents were average of the three parts. The calcium content (355.3 mg/100 g) and zinc content 8.36 mg/100 g of thyme *schimperi L.* Species found in this study were higher than the 9.89 mg/100g and 0.8 mg/100 g reported one of the two by (Ereifeji *et al*., 2010) in the journal of medicinal pant research Vol. 6. On the other hand the iron (0.012 mg/100 g), copper (0.045 mg/100 g) and magnesium (0.21 mg/100 g) contents determined in this work were lower than the 5.0, 0.2 and 71.6 mg/100g reported by (Ereifeji *et al*., 2010).

Table 1. Mineral contents of the raw, wild Abyssinian Thyme herb aerial parts mg/100g

A.P	Zn	Fe	Cu	Mg	Ca
F	9.19±0.1^{a}	0.022±0.01^{a}	0.038±0.01^{c}	2.47±0.01^{a}	392.2±0.1^{a}
L	9.01±0.1^{b}	0.0022±0.001^{c}	0.054±0.01^{a}	2.32±0.01^{b}	379.5±0.4^{b}
W	8.36±0.1^{c}	0.012±0.01^{b}	0.045±0.01^{b}	2.1±0.04^{c}	355.3±0.1^{c}

Data are mean±std of triplicate analysis. Values in a column with the same letter are not significantly different (P>0.05). F= thyme flower- leave, L= thyme leave and W = whole thyme aerial parts, AP = thyme herb aerial parts

3.2. Phytochemicals and anti-nutritional factor of raw Abyssinian thyme plant

The data of phytochemicals and anti-nutritional factor contents of the wild Abyssinian thyme herb are presented in the Table 2 and showed the effect of the aerial parts (flower, leaves and whole thyme) on phytochemicals and anti-nutritional factor contents used in this study. Values exhibited for carotenoid content were 59.6, 68.7 and 63.0 IU, respectively. Similarly, beta carotene of the same parts of herb 119.5, 137.0 and 126.7 IU, while the readings for retinol equivalent vitamin A contents in the same unit amounted to 239.5, 274.8 and 252.0 IU, respectively.

The flower, leaf and the whole thyme parts exhibited anti-nutritional factor contents of condensed tannin of 0.2, 0.4 and 0.9 µg/100 ml and total phenolic content of 0.5, 0.2 and 0.3 µg/100 ml, respectively. From the wild Abyssinian thyme aerial parts the flower had highest total phenolic content and the leaf the lowest and data shows that the whole thyme plant had the highest condensed tannin content of the three aerial parts. The statistical analysis of the data showed that the values of phytochemical and anti-nutritional factor components exhibited significant ($P<0.05$) differences in each of the three plant parts, the leaves showing the highest value of phytochemicals in all three components and the flower showing the lowest values. The condensed tannin content of whole plant showed the highest value (0.9 µg/ml) and the flower showed the lowest value (0.2 µg/ml).

Table 2. Phytochemicals in IU and anti nutritional factors µg/100mL of raw Abyssinian Thyme plant (db)

A. P.	Carotenoids	β-carotene	Retinol E.	C.Tannin	T.Phenolic con.
F	59.6 ± 0.1^{c}	119.5 ± 0.10^{c}	239.5 ± 0.20^{c}	0.2 ± 0.00^{c}	0.5 ± 0.01^{a}
L	68.7 ± 0.01^{a}	137.0 ± 0.10^{a}	274.8 ± 0.01^{a}	0.4 ± 0.01^{b}	0.2 ± 0.00^{c}
W	63 ± 0.64^{b}	126.7 ± 0.20^{b}	252.0 ± 0.10^{b}	0.9 ± 0.01^{a}	0.3 ± 0.00^{b}

Data are mean±std of triplicate analysis. Values in a column with the same letter are not significant differences ($P>0.05$).F= thyme flower, L= thyme leaves and W= whole thyme aerial parts, IU = international unit, µg/ml = microgram per milliliter, A.P= thyme herb aerial parts, retinol E= retinol Equivalent

4. CONCLUSIONS

The present study showed that inorganic contents, phytochemical and anti- nutritional factors are available and they are significantly affected by the Abyssinian thyme herb aerial parts (flower, leaves and whole plant). The condensed tannin and total phenol content are an indication of anti-nutritional factor and are influenced by all the aerial parts of the wild Abyssinian thyme herb.The Abyssinian thyme herb aerial parts (flower, leaf and whole plant) had significantly influenced to the inorganic contents and within these three aerial parts flower parts have the highest inorganic content as compared to the rest.

5. RECOMMENDATIONS

- It is advisable to study the refreshing bioactive compounds and antioxidant contents of the Abyssinian thyme herb aerial parts
- Further studying is needed to investigate essential oil and amino acid contents of the wild Abyssinian thyme aerial parts.

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