

Quantification of Bioactive Constituent D-Pinitol in Ethiopian Soybean

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Received: January 09, 2018; Accepted: January 18, 2018; Published: January 27, 2018

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Abstract

Vegetable soybean (*Glycine max*) is well established legume in the human diet in all over the world. D-Pinitol is a natural product belonging to groups of Cyclitols. D-pinitol plays tremendous medicinal roles. The aim of this work is to quantify the bioactive constituent D-Pinitol from seeds, seed pods and leaves of soybean. D-Pinitol extraction of soybean was processed with methanolic extraction followed by quantification using a UV spectrophotometer in comparisons with the Caro pinitol®. The maximum wavelength and absorbance of the standard dissolved in laboratory reagent water were 193 nm and 0.904 respectively. The best-fit line, R^2 , was found to be greater than 0.9983, indicating the D-pinitol's concentration has significant effect on D-pinitol's absorbance over a concentration range of 31.25-1000.0 µg/mL. The mean recovery of the method was $94.3 \pm 8.06\%$. The absorbance of the serially diluted standard of 31.25 µg/mL, 62.5 µg/mL, 125 µg/mL, 250 µg/mL, 500 µg/L and 1000 µg/mL at 193 nm is 0.032, 0.042, 0.096, 0.205, 0.375, and 0.714, respectively. The absorbance of the samples Nyala seed (so), Nyala seed (mz), Nova seed (so), Nova seed (mz), Nyala seed pod (so), Nova seed pod (so), Nyala leaf (so) and Nova leaf (so) at 193 nm were 0.237, 0.267, 0.307, 0.204, 0.213, 0.276, 0.24 and 0.263, respectively. The quantity (gm) of D-pinitol within 25 gm crude extract of Nyala seed (so), nyala seed (mz), nova seed (so), nova seed (mz), nyala seed pod (so), nova seed pod (so), nyala leaf (so) and nova leaf (so) were 16.2, 18.4, 21.3, 13.9, 14.5, 19, 16.5 and 18.1, respectively. The quantitative values of all varieties are closer to each other and this may be attributed to the similarity between the sampling areas, South Omo and Metekel zone. Generally, the Ethiopian soybean has high D-pinitol content. The pairwise comparison using the Student's t-test shows no significant difference, $p > 0.05$, between the content of D-pinitol in seed, seed pod and leaf.

Keywords: Soybean; D-pinitol, UV spectrophotometer; Quantification; T-test

Acronyms

n.d.	no date
df	dilution factor
so	South Omo
mz	Metekel Zone

Introduction

A compound d-(+)-Pinitol (3-O-methyl D-Chiro inositol) is a natural product belonging to groups of Cyclitols (cyclic polyol) [1].

D-pinitol has been appears to act downstream in the insulin-signaling pathway to mimic the effects of insulin i.e., antidiabetic activities [2-6]. Thus, in humans, this compound may assist with diabetes by lowering blood sugar levels and making the glucose more available to the cells i.e., glucose metabolism [2,7-9] and promoting the glycogen synthesis [2]. Pinitol can also drive creatine and other nutrients into muscle cells [2,10]. Furthermore, an insecticidal effect has been described caused by this cyclitol on the larval growth of *Heliotis zea*, *Aedes egypti* and *Culex quinquefasciatus* [1,11].

It was also found to be responsible for activities of chronic complication obesity; Hyperlipidemia; Dyslipidemia atherosclerosis; Hypertension; malnutrition, stress, aging, Hyperuricemia and

Anthelmintic [1] and more recently, for the ability to modulate the immune response by interacting with dendritic cells (DCs) maturation [12].

It also exerts multifunctional properties, including anti-inflammatory activity [5,13,14], preventing wasting and inflammation in HIV/AIDS patients [6]. It prevents cardiovascular diseases [1,15,16] and used as an antioxidant [15]. Moreover, D-pinitol is an active principle feeding stimulant activities [5].

Animal studies showed that D-Chiro-inositol is synthesized endogenously in small quantities, while in human most D-Chiro-inositol is obtained from dietary pinitol [13].

In plants, this bioactive molecule is an important stress metabolite and its accumulation may be related to plant tolerance to water deficit stress [17]. It occurs in species adapted to temperature extremes, such as jojoba (*Simmondsia chinensis*) [18] and maritime pine (*Pinus pinaster*) [19]. It has been isolated from various plants [2,8,9], trees and citrus fruits [2]. It's found primarily in legumes [2] and is a major component of the soybean plant [20-22]. In soybeans, pinitol has been found in various tissues, including leaf blades, petioles, stems, roots, nodules and seeds [23,24].

Many pharmaceutical preparations of D-pinitol are marketing the popular D-pinitol products under the trade name Biochem GlucoLean® and Inzitol® can help to facilitate glycogen or circulating sugar into metabolically active tissues [5]. Thus, world is now moving towards the natural product derived drugs like D-pinitol that tend to cure diseases without any toxic side effects. Despite of these, to the best of the

authors' knowledge, D-pinitol was yet not investigated from soy bean from Ethiopia. Therefore, the aim of the current study is to extract and quantify D-pinitol, from soybean leaves, seeds, and seed pods.

Methodology

Sample collection

Seed, seed pod and leaf samples were collected from the research fields of Jinka agricultural research center and Assosa agricultural research center from the research fields during harvest season.

Extraction method

The experiment was conducted at applied chemistry laboratory, ASTU, from January 2017 to April 2017.

Seed, seed pod and leaf were ground. Powdered material was kept for soaking in petroleum ether for 24 hours and filtered. The process was repeated three times for residue. Similar soaking was repeated by methanol (Table 1). All filtered portions were combined and evaporated in a Rotavapour to give the brown mass (seed and seed pod extracts) and greenish (leaf extract) at 45-50°C to yield a crude extract residue. The extract keeps in refrigerator for further investigation.

No.	Sample type	Amount of powder used for soaking (gm)	Gram obtained		Pinitol (gm) in 25 gm crud extract	Absorbance at 193nm
			After PEt (500 mL)	After MeOH (500 mL) (solid)		
1	Nyala Seed (So)	100	10.8 (oil?liquid)	5.5	16.2	0.237
2	Nyala Seed (Mz)	100	14.7 (oil?liquid)	7.5	18.4	0.267
3	Nova Seed (So)	100	14.5 (Oil?liquid)	5.5	21.3	0.307
4	Nova seed (Mz)	100	12 (oil?liquid)	5.9	13.9	0.204
5	Nyala seed pod (So)	100	1.8 (solid)	2.3	14.5	0.213
6	Nova seed pod (So)	100	1 (solid)	2	19	0.276
7	Nyala leaf (So)	100	1.4 (solid)	14.7	16.5	0.24
8	Nova leaf (So)	100	2.6 (solid)	16.5	18.1	0.263

Table 1: Pinitol extraction and quantification.

D-pinitol standard optimization and sample dilution

The Caro pinitol[®] (pinitol from carob), Amicogen, Inc., was used as standard. The standard was dissolved in laboratory reagent water for UV spectrometer measurement and the maximum wavelength of the absorbance was recorded (193 nm). Then the standard was prepared with the concentration of 31.25 µg/ml, 62.5 µg/ml, 125 µg/ml, 250 µg/ml, 500 µg/ml and 1000 µg/ml to check the absorbance linearity at 193 nm. The absorbance of the serially diluted standard of 31.25 µg/ml, 62.5 µg/ml, 125 µg/ml, 250 µg/ml, 500 µg/ml and 1000 µg/ml at 193 nm were 0.032, 0.042, 0.096, 0.205, 0.375, 0.714 respectively increases linearly.

The 25 mg of methanol extract was dissolved in 25 ml detergent water for UV spectrometer measurement as a stock solution of each sample. Then 100 µl of the stock solution was mixed with 5 ml of detergent water (i.e. DF=5 ml H₂O+0.1 ml sample/0.1 ml=51).

D-pinitol quantification

Eight samples Nyala seed from south omo, Nyala seed from Metekel zone, Nova seed from south omo, Nova seed from Metekel zone, Nyala seed pod from south omo, Nova seed pod from south omo, Nyala leaf from south omo, Nova leaf from south omo were quantified and compared with the standard as references.

Results and Discussion

D-pinitol standard optimization

The sterile, deionized water was used as solvent as using methanol solvent could not give the clear peak. The lowest wavelength of measurement with the water solvent is 190 nm [25]. The measurement of standards and the samples attained this criterion.

The D-pinitol standard curve was determined using 31.5 µg/ml, 62.5 µg/ml, 125 µg/ml, 250 µg/ml, 500 µg/ml and 1000 µg/ml by serial dilution from a 1 mg/ml pinitol standard. The best-fit line, R₂, was found to be greater than 0.9983, indicating the D-pinitol's concentration has significant effect on D-pinitol's absorbance over a concentration range of 31.25-1000.0 µg/ml (Figure 1). i.e., ~99.8% of the variance in the data is explained by the line and 0.02% of the variance is due to unexplained effects. The mean recovery of the method was 94.3 ± 8.06%. Therefore, the followed method for D-pinitol quantification is precise and accurate.

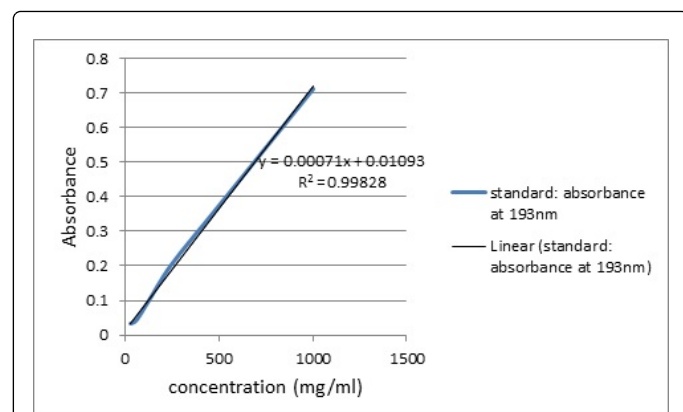


Figure 1: D-pinitol standard absorbance at 193 nm (standard/calibration curve).

D-pinitol standard absorption and wavelength (nm)

The maximum absorption of the D-pinitol standard at 193 nm is 0.904 (Figures 2 and 3). This is the proximate figure with Mininath reported as 229 nm [26]. However, the quoted author used methanol solvent whose lowest wavelength of measurement is 205 nm [25].

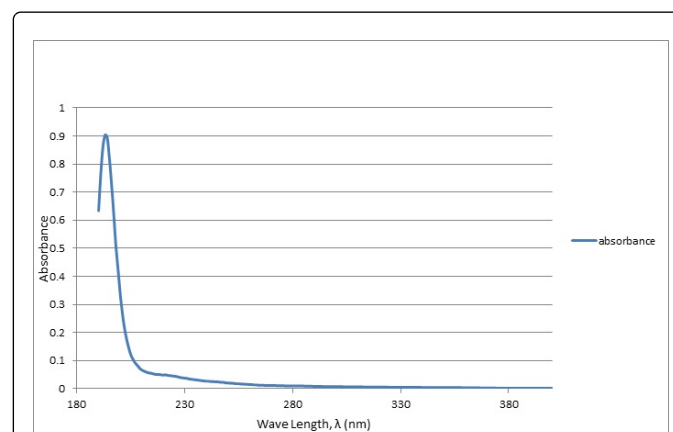


Figure 2: Max absorbance of the D-pinitol standard at 193 nm.

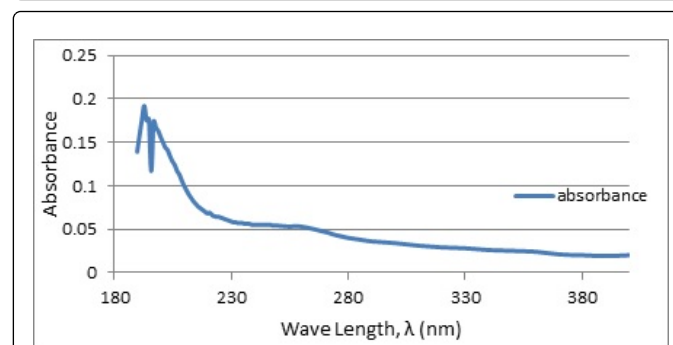


Figure 3: Absorbance of randomly selected representative seed sample (Nova-mz).

Samples' absorption and wavelength (nm)

The absorption of a selected representative sample (Nova seed from Metekel zone) is 0.192 at 193 nm which is proximate to the result of the standard, 0.904.

The concentration and the absorbance of samples

The absorbance of the samples Nyala seed (so), Nyala seed (mz), Nova seed (so), Nova seed (mz), Nyala seed pod (so), Nyala leaf (so) and Nova leaf (so) at 193 nm respectively were 0.237, 0.267, 0.307, 0.204, 0.213, 0.276, 0.24 and 0.263. The concentration of d-pinitol in each sample are calculated using the formula from the trendline equation of $y = 0.00071x + 0.01093$. Accordingly, the quantity (gm) of d-pinitol within 25 gm crude extract of Nyala seed (so), Nyala seed (mz), Nova seed (so), Nova seed (mz), Nyala seed pod (so), Nova seed pod (so), Nyala leaf (so) and Nova leaf (so) is 16.2, 18.4, 21.3, 13.9, 14.5, 19, 16.5 and 18.1 respectively. This concentration range is in agreement with the concentration range of the standard. Therefore, the isolated compound from tissues of soybean leaf, seed and seed pod is D-pinitol. Even though, the variation of concentration of samples from different areas is expected as the accumulation of pinitol is associated with heat stress, drought stress and salt stress [19-21,27-29], the quantitative values of all varieties are almost proximate to each other. This may be attributed to the similarity between the sampling areas, South Omo and Metekel zone.

D-pinitol found in all tissues of soybean, leaf, seed and seed pod. This is in agreement with previous reports, D-pinitol is present in soybean leaves [29,30]; leaves are the major site of pinitol synthesis [18,31,32] and pinitol has been suggested as the principal transport carbohydrate in soybean petiole exudates [17,33]. Soybean plants exposed to high temperature had significantly higher pinitol contents in leaves and stems, and the pinitol content in stems was almost doubled. Pinitol content in soybean leaves, stems and roots are generally in a descending order: pinitol is transported out of the leaves to stems and roots [21,33]. Free D-pinitol concentration is highest in seed coats and lower in the axis and cotyledons of soybean seeds [34], suggesting the transport of D-pinitol from leaves to seeds [35]. The presence of pinitol in tissues other than the seed is important in the matter of using seed for other purposes and getting other tissues is simpler than seed.

Statistical analysis between two samples was performed using the Student's t-test [36]. The pairwise comparison was done in seed, seed pod and leaf samples collected from south omo. In all cases, $p > 0.05$. i.e., no significant difference between the d-pinitol content of seed, seed pod and leaf.

As the sample collected from metekel zone is only a seed, pairwise comparison was not made. Further study is needed to analyze the pinitol content of different tissues of Ethiopian soybean.

Even though, it needs to investigate the relative constituent of D-pinitol with other phytochemicals, the D-pinitol content of the Ethiopian soybean is high as compared to different previous reports conducted other than Ethiopia. For example, Davis reported as Pinitol constitutes 1% of the dry weight of soy meal [2]. Mature and dried soybean seeds contain up to 1% D-pinitol [14,37].

Conclusions and Recommendations

In the present investigation on the basis of UV spectrophotometer data, it was concluded that the isolated compound from the methanolic extract of *Glicine max* is a D-Pinitol. The D-pinitol content of the Ethiopian soybean is high compared to previous reports.

It is the authors' recommendation that different methods are better to be used for further identification and characterization of the D-pinitol from the Ethiopian soybean. More Investigations are needed to address the relationship between the pinitol concentration level and different environments in Ethiopia. It is also very useful if the relative constituent of D-pinitol compared with other phytochemicals found in Ethiopian soybean.

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