

S-adenosyl-L-methionine, trehalose and oleanolic acid in few plants

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ABSTRACT

S-Adenosyl-L-Methionine (AdoMet), S-Adenosyl-L-Homocysteine (AdoHcy), adenosine, trehalose and oleanolic acid were measured in six medicinal herbs and three spices. The findings showed that AdoMet content was forty six fold higher in the leaves of *Catharanthus roseus* as compared with average AdoMet content of rest of the plants. In comparison to other plants, *Withania somnifera* had very high AdoHcy: AdoMet and adenosine: AdoMet ratios indicating it may have contained high AdoMet. Trehalose was found to be twenty fold and nine fold higher in bulb of *Allium cepa* and root of *Withania somnifera* respectively with respect to average trehalose content of rest of the plants. *Ocimum sanctum* appeared to be a rich source of oleanolic acid. It appeared from our study that *Catharanthus roseus*, *Allium cepa* and *Ocimum sanctum* could be utilized as natural sources of AdoMet, trehalose and oleanolic acid respectively.

Keywords: AdoMet; AdoHcy; Adenosine; Trehalose; Oleanolic Acid

1. INTRODUCTION

S-Adenosyl-L-Methionine (AdoMet) is the only natural sulfonium compound present in different living organisms. It is an important metabolic intermediate participating in different biochemical events and acts as a universal methyl group donor in transmethylation reactions, transsulfuration reactions and polyamine synthesis [1]. The molecule has therapeutic importance in the treatment of alcoholic liver disease [2], cirrhosis of liver [3], depressive syndrome [4], Alzheimer disease [5], Osteoarthritis [6] etc. and may be classified as an “anti-aging compound”.

Trehalose (α -D-glucopyranosyl- α -D-glucopyranoside) is a non reducing disaccharide having high glass transition temperature ($T_g = 80^\circ\text{C}$) is well known for effective stabilization of macromolecules like proteins [7], small molecules like AdoMet [8], etc. It also acts as a cryopreservative of cellular membranes [9].

Oleanolic acid is a triterpenoid compound that exists widely in food, medicinal herbs and other plants. It is effective in protecting against chemically induced liver injury in laboratory animals where the mechanism of hepatoprotection may involve the inhibition of toxicant activation and the enhancement of the body defense systems [10]. Oleanolic acid has also been long recognized to have antiinflammatory and antihyperlipidemic properties in laboratory animals [11]. But more research is warranted to develop a therapy for patients. Recently, oleanolic acid has been noted for its anti-tumor activity [12].

Extracts from the leaf of *Ocimum sanctum* [13], stem of *Tinospora cordifolia* [14], rhizosphere of *Picrorrhiza kurroa* [15], root of *Withania somnifera* [16], whole body (except root) of *Eclipta erecta* [17], bulbs of *Allium sativum* [18] and *Zingiber officinale* [19] are already proven for their hepatoprotective activities beside other therapeutic potentialities.

In the present communication, we have made comparative studies on AdoMet, trehalose and oleanolic acid content among the above mentioned medicinally important plants. For our experiments we have taken root of *W. somnifera*, leaf of *O. sanctum*, stem of *T. cordifolia*, rhizosphere of *P. kurroa*, whole body (except root) of *E. erecta* and bulbs of *A. sativum* and *Z. officinale*. The bulb of *Allium cepa*, which is well known for its food preservation activities and leaf of *Catharanthus roseus* [20] which have anti-tumor, anti-oxidative activities were also chosen for analysis.

2. MATERIALS AND METHODS

The experiments were performed in triplicate by col-

lecting different parts of young herbs and spices from a particular area of eastern India in winter season so that within different experimental sets geological, environmental and chronological variations could be avoided. The intracellular matter from the specific parts of the different herbs and spices were simultaneously extracted and deproteinized [21] prior to the estimation of trehalose, AdoMet and its irreversibly transformed metabolic products AdoHcy and adenosine. Briefly, each of the herbs and spices were cut into small pieces and washed thoroughly with tap water.

3. EXTRACTION AND DEPROTEINIZATION

The washed pieces were then air-dried and 0.25 g (dry weight) of each of the plant parts was homogenized with 0.5 ml of cold 0.5 N perchloric acid for deproteinization purpose. The mixture was centrifuged at $5,500 \times g$ for 10 min and the pellet was extracted further with 0.5 ml of cold 0.2 N perchloric acid and again centrifuged. The process was repeated and the supernatants were pooled. The pH value of the pool was adjusted to 4.5 by careful addition of 3 N KOH. This caused precipitation of potassium perchlorate and the precipitate was removed by low speed centrifugation for 10 minutes at 4°C. Careful reduction of volume under reduced pressure at relatively low temperature resulted in further precipitation of potassium perchlorate and this was again removed by low speed centrifugation. The supernatants were dried and reconstituted with 1ml of 0.01 N HCl.

4. ESTIMATION OF ADOMET, ADOHCY AND ADENOSINE

AdoMet, AdoHcy and adenosine content of different herbs and spices were quantitatively estimated by cation exchange High Performance Liquid Chromatography (HPLC) [22]. The HPLC column used was Partisil 10SCX, 4.6×250 mm, Whatman Inc., England, fitted to an HPLC system consisting of two pumps (Model 515), a Rheodyne injector and a programmer controlled by pump control module (PCM). Elution of AdoMet was monitored in-line by measurement of absorbance at 259 nm (A_{259}), using a dual wavelength UV-Visible detector (Model 2487). Elution times as well as peak quantification were obtained from the Millennium³² software. All these HPLC equipments were from Waters, USA.

AdoMet, AdoHcy and adenosine were eluted near 31.617 min, 10.998 min and 8.993 min. The amounts of standard compounds AdoMet, AdoHcy and adenosine (expressed in μg) were plotted along the x-axes and respective peak areas were plotted along the y-axes and compared with the samples (in triplicate) from different

plant extracts. The standard solutions of AdoMet (Sigma, USA), AdoHcy (Sigma, USA) and adenosine (Sigma, USA) were prepared by dissolving in 0.01 N HCl.

Different volumes (5, 10, 20 μl) of the standard compounds of known concentrations (0.435 mM AdoMet, 2.17 mM AdoHcy and 0.9 mM adenosine) were injected into the HPLC system to get standard curves. Values obtained for test sample (in triplicate) were derived from the standard curve when r^2 (square of correlation coefficient) values were near 0.99.

The amount of trehalose present in the samples was estimated using acid trehalase (AT) enzyme, purified in our laboratory according to published method [23,24]. The standard aqueous trehalose (Sigma USA) solution (1 mg/ml) was prepared. Values represented here are average of experiments done in triplicate.

Oleanolic acid was estimated by Thin Layer Chromatographic (TLC) method [25] with some modifications. Different volumes (2.5, 5.0, 10.0 μl) of standard oleanolic acid (0.9 mg/ml chloroform; Sigma, USA) were spotted on a pre-coated alumina TLC plate along with different plant extracts (1 g dried powder/ml chloroform), 10 μl were spotted on different lanes. The optimized solvent system for best separation of oleanolic acid from the other chemical constituents present in chloroform extract of the different plants was the combination of benzene, chloroform and ethyl acetate in the ratio of 6: 3: 1. After complete air-drying of the TLC plate, it was sprayed by the anisaldehyde-sulfuric acid reagent for derivatization and the plate was immediately taken to a heating chamber (100°C) for 5.0 min and scanned in a HP scanjet (Model # 4570c) scanner at a resolution of 600 dpi and densitometrically quantified by a NIH (National Institute of Health, USA) make software, namely "ImageJ". Different amounts of oleanolic acid were plotted along the x-axis and the respective total pixel counts (pixel density \times area of the spot ($R_f = 0.22$)) were plotted along the y-axis to get a standard curve. Putting the values of total pixel counts on the standard curve the amounts of oleanolic acid present in different plant parts were estimated. The identification of oleanolic acid was done by mass spectrometry after scraping the spot in the TLC plate co-linear with the spots of standard oleanolic acid using ESI-MS analysis in a LC-QTOF system, Micromass, UK and ^1H NMR (300 MHz) study was performed on a DPX 300 NMR instrument, Bruker, Germany using tetra methyl silane (TMS; Sigma, USA) as internal standard.

5. RESULTS AND DISCUSSION

Among the herbs and spices studied, *C. roseus* leaves contained highest quantity of AdoMet (1.63 mg/g) (Ta-

ble 1). Moderate to low AdoMet has been found in the descending order of *O. sanctum* > *A. sativum* > *T. cordifolia* > *W. somnifera* > *A. cepa* > *P. kurroa* > *Z. officinale* > *E. erecta*. *C. roseus* was not only rich in AdoMet but this plant also had highest AdoHcy (0.347 mg/g leaf) and adenosine (0.084 mg/g leaf), the metabolic products of AdoMet. The rest of the plants had either low or moderate quantities of AdoHcy which decreased in the order of *W. somnifera* > *A. sativum* > *O. sanctum* > *A. cepa* > *A. sativum* > *E. erecta* > *T. cordifolia* > *Z. officinale*. No AdoHcy and adenosine were detected in *P. kurroa* and no adenosine was detected in *E. erecta* under the assay conditions. Incidentally, these plants had little AdoMet as well. Besides *C. roseus* the decreasing order of adenosine content was *W. somnifera* > *A. sativum* > *A. cepa* > *O. sanctum* > *T. cordifolia* > *Z. officinale*.

Among the nine plants both *A. cepa* and *W. somnifera* had relatively higher trehalose content (101.94 mg/g bulb and 45.08 mg/g root respectively). Moderate to low amount of trehalose was present in the rest of the plants and decreased in the order of *P. kurroa* > *C. roseus* > *T. cordifolia* > *Z. officinale* > *A. sativum* > *O. sanctum* > *E. erecta*.

O. sanctum appeared to be the only plant which contained oleanolic acid (10.2 mg/g leaf) (blue spot at $R_f = 0.22$) among all the herbs and spices studied.

The leaf of *C. roseus* is well known for the storage of alkaloids like vinblastine and vincristine, which have anti-tumor activities [26]. Through this study another bioactive product of *C. roseus* leaf viz. AdoMet has been detected. Leaves of *C. roseus* contained nearly forty-six fold higher AdoMet in comparison to average AdoMet content of the rest of the plants. Genetically modified *Kluyveromyces lactis* mutants have been recognized as a

good source of AdoMet [27]. Similar genetic manipulations could be performed to get mutant *C. roseus* plants rich in AdoMet. Although *W. somnifera* contained very little amount of AdoMet (0.037 mg/g leaf) but it had both higher AdoHcy (0.148 mg/g root) and adenosine (0.0508 mg/g root) content and the AdoHcy: AdoMet and adenosine: AdoMet ratios were remarkably high compared to other plants. So, it could be said that in *W. somnifera*, although AdoMet was synthesized in appreciable quantities but was mostly utilized for its own metabolic purposes. Both *A. cepa* and *W. somnifera* had twenty fold and nine fold higher trehalose content respectively compared to average trehalose content of the rest of the seven plants and these two plants could be exploited as alternative sources of trehalose as their trehalose content is comparable with other well known trehalose rich sources like yeast (10-15% wt/dry wt) [28], fungi (7-10% wt/dry wt) [29,30], etc. Occurrence of trehalose was reported to form a glassy state in the cell, in which degradative reactions and intracellular ice formation could sufficiently be arrested during prolonged storage. In India, bulb of *A. cepa* is one of the daily used food ingredients. This is the first report of *A. cepa* bulbs containing such a huge percentage of trehalose. Since it is an economically favorable crop and its production rate is also very high, more trehalose enriched genetically improved *Allium cepa* could be cultivated in near future.

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Table 1. Estimation of AdoMet, AdoHcy, Adenosine, Trehalose and Oleanolic acid.

Names of the Plants	AdoMet (% wt/wt) $\times 10^{-4}$	AdoHcy (%, wt/wt) $\times 10^{-4}$	Adenosine (%, wt/wt) $\times 10^{-4}$	Trehalose (%, wt/wt) $\times 10^{-2}$	Oleanolic acid (%, wt/wt)
<i>Tinospora cordifolia</i>	51.37 \pm 0.21	0.90 \pm 0.014	5.50 \pm 0.0076	59.10 \pm 0.06	n.d.
<i>Picrorrhiza kurroa</i>	9.18 \pm 0.03	n.d.	n.d.	99.0 \pm 0.06	n.d.
<i>Eclipta erecta</i>	6.36 \pm 0.05	1.03 \pm 0.006	n.d.	1.01 \pm 0.01	n.d.
<i>Ocimum sanctum</i>	79.68 \pm 0.30	7.73 \pm 0.01	6.83 \pm 0.01	17.05 \pm 0.03	1.02 \pm 0.02
<i>Allium cepa</i>	21.52 \pm 0.17	4.78 \pm 0.08	7.08 \pm 0.02	1019.43 \pm 0.13	n.d.
<i>Zingiber officinale</i>	6.58 \pm 0.05	0.36 \pm 0.01	2.22 \pm 0.01	59.03 \pm 0.06	n.d.
<i>Allium sativum</i>	69.38 \pm 0.20	1.76 \pm 0.01	25.34 \pm 0.15	38.11 \pm 0.03	n.d.
<i>Withania somnifera</i>	37.29 \pm 0.23	148.47 \pm 0.35	50.82 \pm 0.12	450.77 \pm 0.19	n.d.
<i>Catharanthus roseus</i>	1630.42 \pm 0.23	346.77 \pm 0.43	448.29 \pm 0.19	84.40 \pm 0.29	n.d.

n.d. — not detected

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