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Secondary Phenol Metabolites (SPhMs), Distribution and Content of Some *Aloe* Species, Originated from Arid Zones of South Africa: A Review

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Abstract: This review is concentrated on internal and external factors affecting the distribution of Secondary Phenol Metabolites (SPhMs) in the succulent leaves of *Aloe* species as follows: Appearance of a few of SPhMs together in the same leaf in different tissues, as a defense strategy, quantitative distribution of SPhMs in the different parts of the leaves as a peripheral defense strategy, distribution of SPhMs in the different parts of the succulent leaves of *Aloe* plants species according to their: age, location and orientation, content of SPhMs in renewed leave parts after leaf pruning according to their: age, location, direction, orientation and portion of leaf pruning - as a peripheral defense strategy, leaf exudates and leaf water extract of *Aloe* plant, classification of *Aloe* species, using the anthrone-C-glycosides as a biochemical marker and In the conclusions was summarized the main findings mentioned in this review. This study has been done on more than 100 Aloe species originated from the desert of South Africa. These plant species were introduced during the last 20 years, in the Garden of the Jacob Blaustein Institutes for Desert Research, at Sede Boker in the Negev Desert of Israel (34° 46E 30° 51'N, 460 m, asl). The *Aloe* plants have flourished in the Negev desert conditions when planted in loess soil. They are wetted by an annual average of 100 mm of rain in this area in winter; in addition they are irrigated by about 300-400 mm water per year. The aim of this review is to summarize about 15 years of our scientific investigations on the SPhMs content in the succulent leaves of *Aloe* plants species as well as to study agro-technical methods in order to increase the SPhMs content under different treatments. These results were also compared and related to other publications.

Key words: Secondary Phenol Metabolites (SPhMs), *Aloe arborescens*, *A. hereroensis*, *A. mutabilis*, quantitative distribution, renewed leave parts, anthrone-C-glycosides, leaf exudates, leaf water extract

INTRODUCTION

The genus *Aloe* contains about 400 species (Reynolds, 2004). The majority of these plant species are desert plants which inhabit in the Deserts of South Africa. Some of these species are tall trees in size of 5 m or more, while the majority is shrubs 0.5 to 1.5 m tall. Some plants species are very small, measuring only a few cm (Van Wyk and Smith, 1996). More than a 100 Aloe species among the 400

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mentioned earlier, were introduced during the last 20 years, in the Garden of the Jacob Blaustein Institutes for Desert Research, at Sede Boker in the Negev Desert of Israel (34° 46'E 30° 51'N, 460 m, asl).

Aloe plants have long been the source of important products due to their nutritional and therapeutic values. Their leaf exudates are used to a great extent in traditional medicines (Van Wyk *et al*., 1997). In recent years, there has been a growing interest in *Aloe* products for cosmetics, medicine and health foods. *Aloe vera* (*Aloe barbardensis*), *A. ferox and A. arborescens* are plant species used in cosmetics and medicine as powder or exudates. Whole leaves of *A. arborescens* can be used as fresh food (Shioda *et al*., 2003). According to some studies, *A. arborescens* is richer than *A. vera* in respect to medicinal properties. The leaves of *A. arborescens* have long been used externally for therapeutic and cosmetic purposes. Experimentally, it has been demonstrated to exert a number of pharmacological effects (Suga and Hirata, 1983).

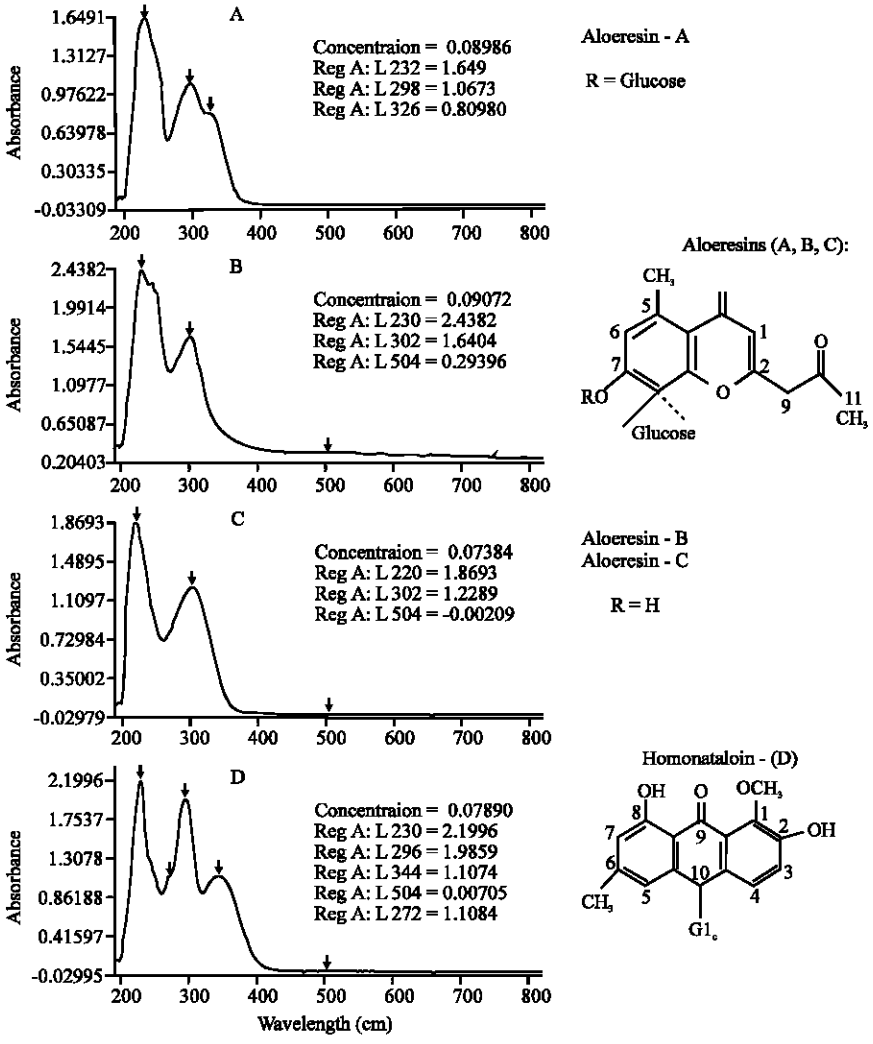

Fig. 1a: The absorption spectrum and the molecular structures of the aloeresin isomers (A, B, C) and homonataloin (D) (Shen *et al*., 2001)

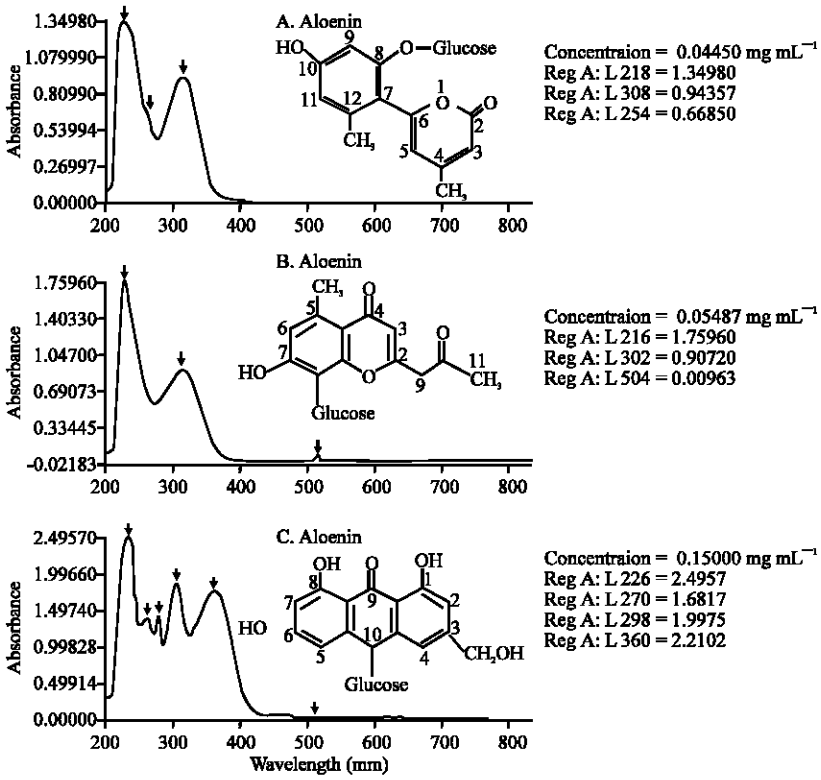

Fig. 1b: Structural formula and absorption spectrum of secondary phenolic metabolites (SPhMs) (A) aloenin, (B) aloeresin and (c) barbaloin, at the wavelength range from 190 to 820 nm in MeOH (mg mL^{-1}). The concentrations (mg mL^{-1}) and absorptions peaks are defined (Gutterman and Chauser-Volfson, 2000b)

The succulent leaves of *Aloe* species have been used for medicinal purposes, cosmetic and food supplement and also in gardening as ornamental plants to save water (Reynolds, 2004).

Among the succulent leaves of *Aloe* plant species, were analyzed anthraglycosides such as: barbaloin, homonataloin, nataloin and phenol derivatives of aloeresins and aloenins (Beaumont *et al*., 1984; Suga and Hirata, 1983; Dagne *et al*., 2000). Barbaloin has been found to have a strong inhibitory effect on the histamine release from mast cells, while aloenin has a weak inhibitory effect. The inhibitory effect of barbaloin is much higher than that of a potent anti-inflammatory drug, such as Indomethaacin (Nakagomi *et al*., 1987). These researches indicate that aloenin and barbaloin have different active sites at the level of the mast cells; Barbaloin demonstrates anti-inflammatory and cathartic effects *in vivo* (Nakagomi *et al*., 1985).

Barbaloin and homonataloin are widely distributed in *Aloe* species, however, nataloin (Conner *et al*., 1987) is a rare anthrone C-glycoside and only two *Aloe* species from our collection contain this phenol metabolite (Chauser-Volfson and Gutterman, 2006). Anthroglycosides, aloeresins and aloenins are considered to be the most specific secondary products in TLC screening of *Aloe* plants. The TLC analysis was used to separate and determine the different phenol metabolites (Hirata and Suga, 1978; Speranca *et al*., 1985; Gutterman and Chauser-Volfson, 2000a).

Secondary metabolites are very important defending compounds against leaf eater in order to keep the natural balance in the green world. It is well known that in the green plants, synthesis takes place from inorganic substances creating organic compounds which are necessary for leaf eaters. The plants synthesize the complicated substances from carbon dioxide gas, water and a variety of inorganic compounds, for plant defense, such substances are defined as secondary metabolites. Terpenoids, alkaloids and a large group of phenols are included in the category of secondary metabolites (Hartly and Jones, 1997).

Distribution of phenol metabolites in leaves seems to relate to defense strategies of a plant. The plant's SPhMs, are defending against insect herbivores (Hartly and Jones, 1997). In addition to defense against herbivores, the phenol metabolites in *Aloe* plants are also involved in the protection of the plants from UV radiation damage (Bennet and Wallsgrove, 1994; Lee *et al*., 1997; Reynolds and Dweek, 1999; Strickland *et al*., 1994). As the top part of the leaves is more exposed to the sun, the higher is the concentration of the secondary metabolites, as occurs with the younger leaves. The appearance of three or four SphMs in one plant may increase the protection efficiency against UV radiation as well as against leaf eaters. In one leaf the different SphMs appear in different tissues and content (Fig. 1-7) (Gutterman and Chauser-Volfson, 2000a, b; Shen *et al*., 2001; Chauser-Volfson *et al*., 2002).

The aim of this review is to summarize the different studies and the different methods, which were used in order to locate and increase the content of secondary phenol metabolites in the succulent leaves of some *Aloe* plant species.

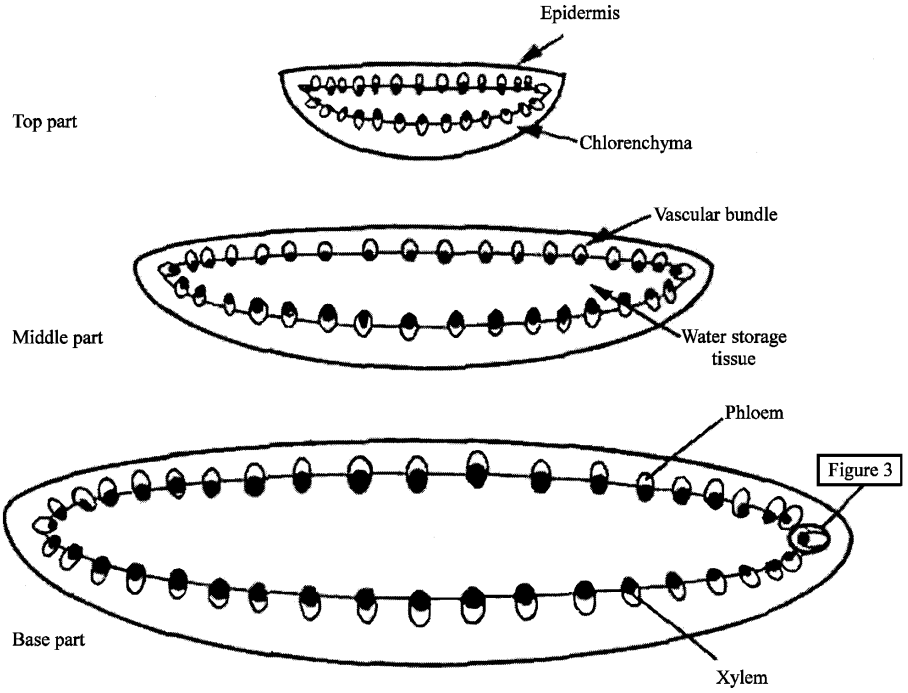

Fig. 2: Cross section of the *Aloe hereroensis* leaf, showing the different density of vascular bundles and width of the chlorenchyma near the top, in the middle and in the basal part of the leaf (Chauser-Volfson *et al*., 2002)

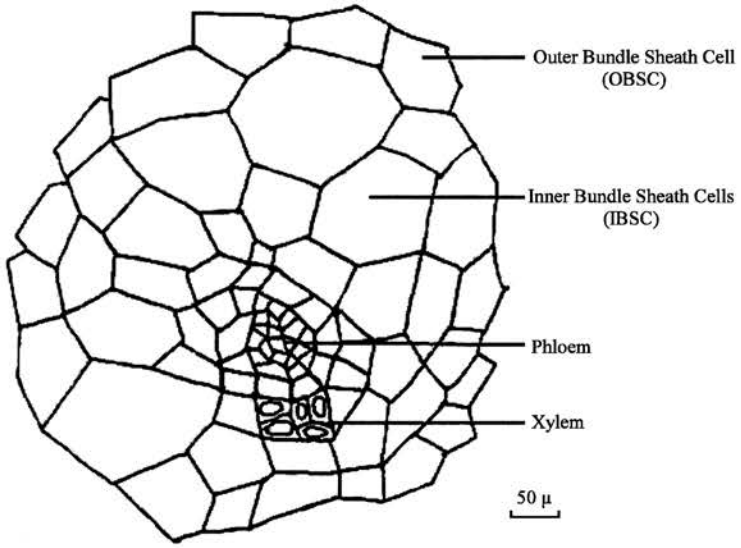

Fig. 3: One part of the cross section in *Aloe hereroensis* leaf, showing the vascular bundles in the adaxial center part (Chauser-Volfson *et al*., 2002).

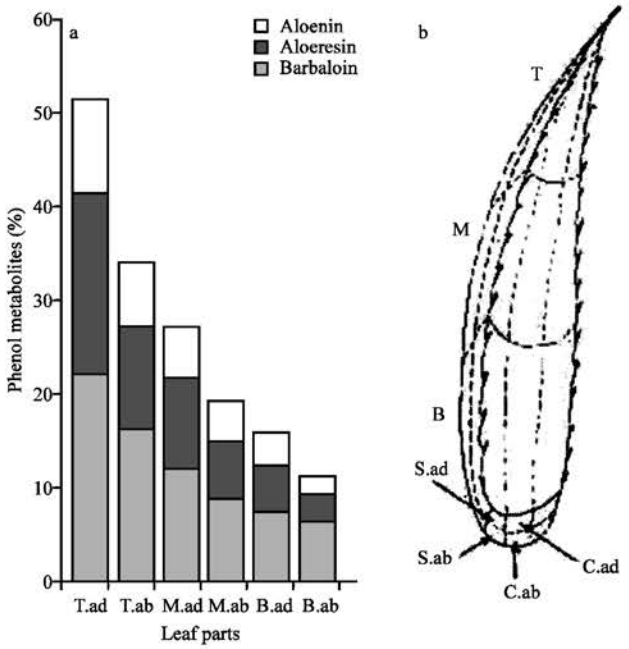

Fig. 4: (a): Average content (%) of aloenin, aloeresin and barbaloin of dry weight of leaf exudate of four *Aloe arborescens* leaves according to section: T = top, M = middle, B = base; ad = adaxial orientation, ab = abaxial orientation. 4b. An upright *Aloe arborescens* leaf cut into three parts: T = terminal, M = middle, B = base These parts were cut into longitudinal parts: c.ab. = central abaxial orientation c.ad. = central adaxial orientation, s.ab.=side abaxial orientation and s.ad = side adaxial orientation (Gutterman and Chauser-Volfson, 2000b)

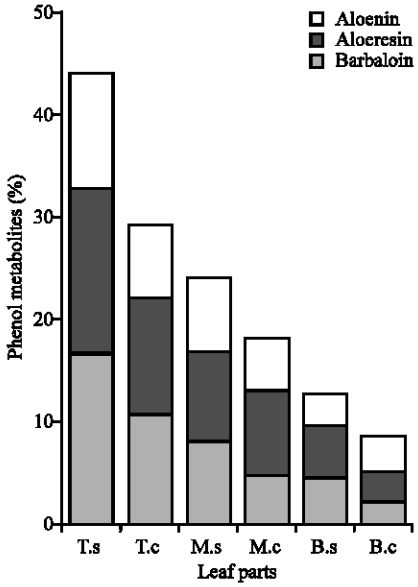

Fig. 5: Average aloenin, aloerisin and barbaloin content (%) of dry weight of five upright bending leaves of *Aloe arborescens* (Gutterman and Chauser-Volfson, 2000b)

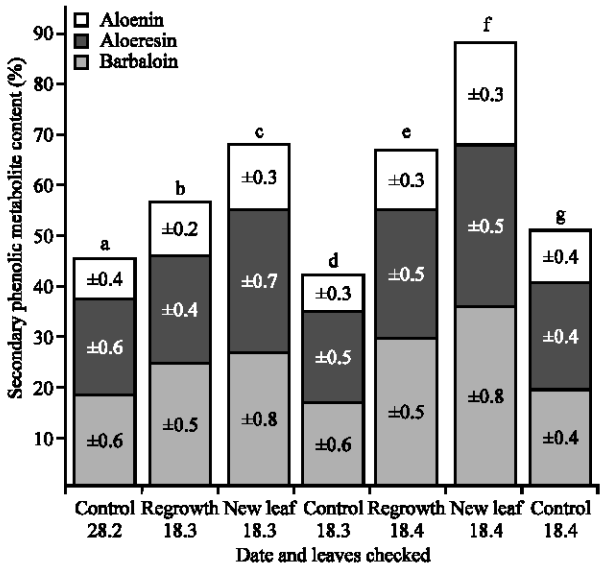

Fig. 6: The influence of leaf pruning on the average content (% ±SE) of barbaloin, alanine and aloerisin in the dry weight of exudates of newly developed leaves at the top of branches from four *Aloe arborescens* plants, compared with the content of the regrown parts of the previously pruned leaves. On 18 March 2001 (b) in renewed growth of leaves 2, 3 and 4 and (c) in the new leaves 2,3 and 4 at the top of the same branches of the Aloe plants, (d) in control of the leaves 2, 3, 4 in previously unpruned plants. On 18 April (e) in renewed growth of leaves 2, 3, 4 of (b); (f) in new leaves 2, 3 and 4 of previously unpruned plants (Chauser-Volfson and Gutterman, 2004)

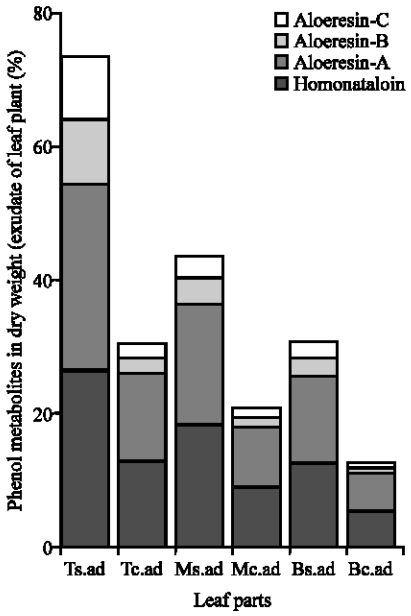

Fig. 7a: Percentage of SPhMs in dry weight exudate in leaf parts of *Aloe hereroensis*; Ts.ad = top side adaxial part, Tc. Ad. = top center adaxial part, Ms.ad.=Middle side adaxial part, Mc.ad. = Middle center adaxial part, Bs.ad = Base side adaxial part, Bc.ad. = Base center adaxial part (Chauser-Volfson *et al*., 2002)

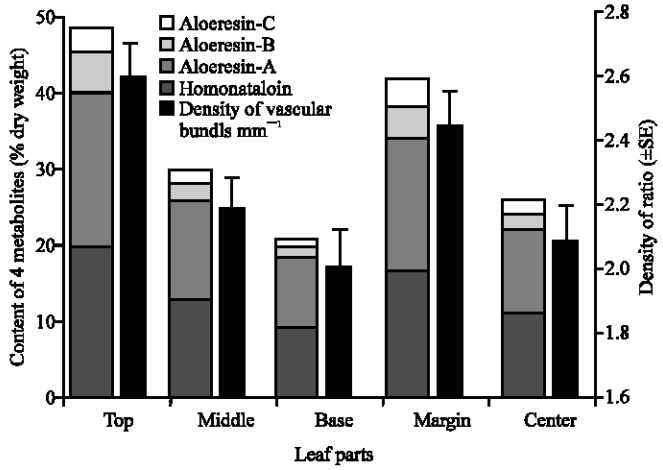

Fig. 7b: Four SPhMs as % of the dry weight in leave parts of *Aloe hereroensis* and as well as the density of vascular bundles in the leaf parts (Chauser-Volfson *et al*., 2002)

APPEARANCE OF A FEW OF SECONDARY PHENOL METABOLITES (SPhMs) TOGETHER IN THE SAME LEAF IN DIFFERENT TISSUES, AS A DEFENSE STRATEGY

The different SPhMs (Fig. 1a, b) (Shen *et al*., 2001; Gutterman and Chauser-Volfson, 2000), was found to appear in different colors when they were separated on TLC and illuminated by UV light.

When a slide of a living tissue of the Aloe succulent leaf were observed under a microscope illuminated by UV light, the different SPhMs appears in different colors in different tissues of the same leaf. Each of the SPhMs appeared in its own color, when they were located in the living tissues of the leaf.

The typical structure of the cross section in the succulent leaves of the Aloe plant species is a composition of water storage tissue (the gel) which is shrouded by a green tissue (chlorenchyma). Between these two tissues are located the vascular bundles (Fig. 2). In the cross section of a vascular bundle there exist an outer bundle sheath cells and an inner bundle sheath cells which surrounds the Phloem and the Xylem tissues (Fig. 3). By testing anatomic cross sections of *Aloe hereroensis* leaves under fluorescence microscope, it was found that: homonataloin accumulates mainly in the inner bundle sheath cells and the three aloeresin isomers, accumulates mainly in the outer bundle sheath cells (Fig. 3), (Chauser-Volfson *et al*., 2002).

In some of the *Aloe* plant species there can be found 3 or 4 different SPhMs in the same leaves. The relative content of each of the different SPhMs in the particular parts of the leaves is distributed proportional to the total content of the SPhMs.

In *Aloe arborescens*, which contain three SPhMs, the content of barbaloin or aloeresin was found to be the highest and the content of aloenin was found to be the lowest. (Fig. 4-6) (Gutterman and Chauser-Volfson, 2000a, b; Chauser-Volfson and Gutterman, 2004).

In *A. hereroensis* leaves, which contain four SPhMs, the aloeresin isomer A content was found to be the highest, followed by content of homonataloin. The content of the two aloeresin isomers B and C was found to be the lowest (Fig. 7) (Chauser-Volfson *et al*., 2002).

The fact that a few of different SPhMs may appear together in the same leaf is an important defense strategy (Chauser-Volfson *et al*., 2002; Chauser-Volfson and Gutterman, 2004; Gutterman and Chauser-Volfson, 2000a, b).

QUANTITATIVE DISTRIBUTION OF SECONDARY PHENOL METABOLITES (SPhMs) IN THE DIFFERENT PARTS OF THE LEAVES AS A PERIPHERAL DEFENSE STRATEGY

The distribution of SPhMs in the different parts of the leaves was found to be different. In the upper part of the leaf, the content of the SPhMs is the highest and in the basal part, the lowest (Fig. 4). Along the leaf margins the content of the SPhMs is much higher than in the leaf center (Fig. 5 and 7). The highest amounts of three SPhMs are found along the margins of the upper third of the leaf and the lowest at the centre of the leaf base as was found in *Aloe arborescens.* (Gutterman and Chauser-Volfson, 2000 a, b). A similar relative distribution of four SPhMs was found in *A. hereroensis* leaves (Fig. 7). As was mentioned above, it was found that: homonataloin accumulates mainly in the inner bundle sheath cells and the three aloeresin isomers, accumulates mainly in the outer bundle sheath cells; therefore the higher the density of the bundle sheath cells the higher is the content of the SPhMs (Fig. 7b) (Chauser-Volfson *et al*., 2002).

DISTRIBUTION OF SPhMS IN THE DIFFERENT LEAVES OF *ALOE* PLANTS SPECIES ACCORDING TO THEIR: AGE LOCATION AND ORIENTATION

The content of the SPhMs in the youngest leaf that is located at the uppers part of the brunch is the highest. From leaf number two (weight of 6-8 g) the lower and older the leaf, the lower is the content of the SPhMs. This was found in *A. arborescens* as well as in *A. hereroensis* (Fig. 8). (Chauser-Volfson and Gutterman, 1996, 1997).

The orientation of the leaf has also an influence on the content of the SPhMs (Gutterman and Chauser-Volfson, 2000a).

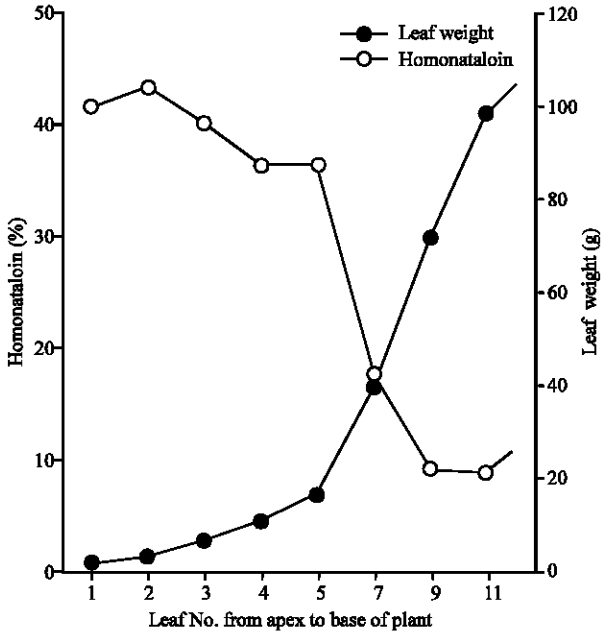

Fig. 8: Homonataloin content (%) as well as leaf weight (gr) in a number of *Aloe hereroensis* leaves from apex to base (Chauser-Volfson and Gutterman, 1997)

CONTENT OF SPhMs IN RENEWED LEAVE PARTS AFTER LEAF PRUNING ACCORDING TO THEIR: AGE, LOCATION, DIRECTION, ORIENTATION AND PORTION OF LEAF PRUNING - AS A PERIPHERAL DEFENSE STRATEGY

In *Aloe arborescens*, the content of the barbaloin (phenol metabolites) was found to increase in renewed leaf parts after pruning. The more times the renewed parts of the leaves were pruned the higher was the barbaloin content in these renewed parts of the leaves (Fig. 9) (Gutterman and Chauser-Volfson, 2000a).

The repeated pruning of parts of a plant during a long time may increase the content of the SPhMs up to 85% of the leaves dry weight (Chauser-Volfson and Gutterman, 2004).

The pruning of even one leaf may have an influence on the content of the SPhMs in the other leaves of the plant.

Leaf number 2 at the top of the brunch on the north side, were pruned. After 38 day, the 5 leaves oriented on the north side, below the pruned leaf were tested. The younger and closer the leaf to the pruned leaf, the higher was the content of the SPhMs. This in compare to the leaves in the control untouched plants that were pruned and tested for their SPhMs content (Fig. 10). After 29 days the re-grown parts of these pruned 5 leaves were checked again and it was found that the younger the leaf the higher is the SPhMs content. The influence of pruning is effective also in laves located at the opposite side of the pruned laves. They respond at lower levels but in relative amounts. This in comparison to leaves at the same direction but in the control untouched plants (Fig. 10) (Chauser-Volfson and Gutterman, 2004).

Aloe arborescens levels were pruned to 25, 50, 75 and 100% of the leaf on 22.10.2003 and the regrown leaf parts were tested fore their content of SPhMs at 23.11.2003,

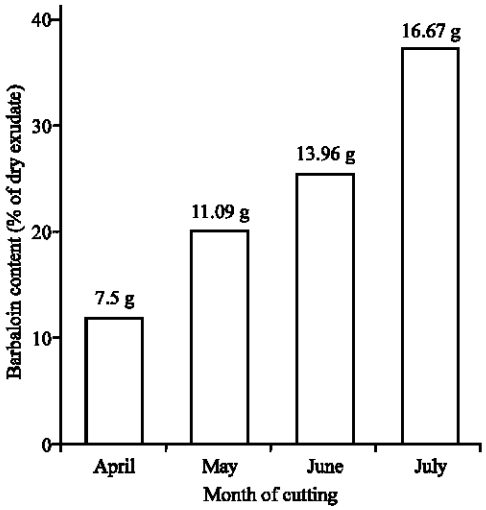

Fig. 9: Barbaloin content as a percentage of the dry weight of an *Aloe arborescens* leaf cut on 27 April 1993 and the consequent new growths from the same place on the plant cut on 27 May, 27 June and 27 July. The number above the column is the weight of the cut leaf (Gutterman and Chauser-Volfson, 2000a)

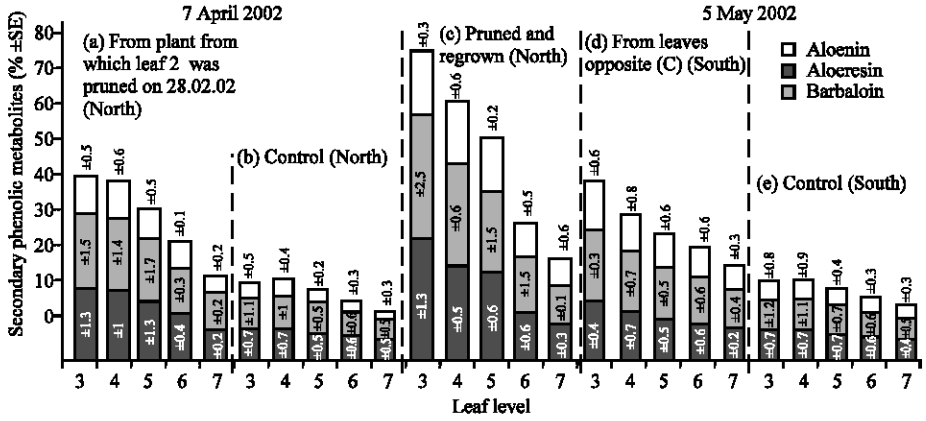

Fig. 10: The influence of pruning leaf level 2 on the average content (% ±SE) on the three SPhMs, aloenin, aloeresin, barbaloin in dry weight of exudates of *Aloe arborescens* leaves. (a) On the 28 February 2002, leaf level 2, at the top of the branches from four plants were pruned. On 7 April 2002 (38 days later) north-facing leaf levels 3-7 from the same branch were pruned and tested, (b) On 7 April 2002, north-facing leaf levels 3-7 of the branches from four previously untouched plants were pruned and tested as the control, (c) On 5 May 2002(after 29 days) the pruned and regrown north-facing leaves on a branch B were pruned and tested, (d) On 5 May 2002 south-facing leaf levels 3-7 opposite the pruned and regrown north-facing leaf levels 3-7 pruned from branches of treatment C were pruned and tested and (e) On 5 May 2002 south-facing leaf levels 3-7 from four previously untouched plants were pruned and tested as a control (Chauser-Volfson and Gutterman, 2004)

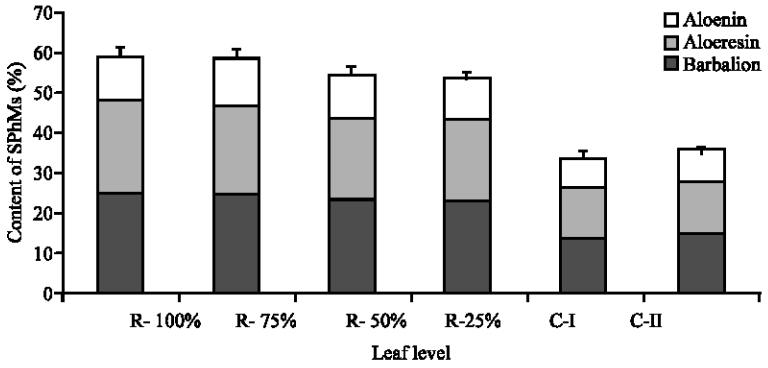

Fig. 11: In *Aloe arborescens*, a cut of 25, 50, 75 and 100% of the leaf No. 10 resulted in different content of SPhMs (barbaloin, aloeresin and aloenin) in the regrown leaf parts. The levels were pruned on 22.10.2003 and the regrown leaf parts were tested for their content of SPhMs at 23.11.2003, (Chauser-Volfson and Gutterman, 2005)

In the regrown leaf parts of the levels that were pruned to 75 and 100%, it was found a higher % content of SPhMs, than in regrown leaf parts of the levels that were pruned to 25 and 50%. In the control plants it was found a much lower % of SPhMs content than in the pruned and the regrown leaf parts (Fig. 11) (Chauser-Volfson and Gutterman, 2005).

LEAF EXUDATES AND LEAF WATER EXTRACT OF *ALOE* PLANT

The SPhMs were separated from pruned leaves of the same *Aloe arborescens* plants at the same time by two methods: (1) Exudation by squeezing the tissues of the top, middle and base leaves parts and (2) Immersion of the pruned cut bottom leaf parts in water and collection of the extract. The exudates and extract were frozen and freeze-dried to a powder. Then the SPhMs were separated by chromatography.

The yield of SPhMs (barbaloin aloeresin and aloenin) of the pruned leaves in the powder from water extraction was between 80 to 93%. The content of SPhMs in leaf exudates from pruned leaves was much lower, between 39-62% (Fig. 12).

Therefore, the use of water extract method is the more efficient method in order to obtain *Aloe arborescens* powder with the highest SPhMs content. Furthermore, this method represents an easier way to separate these compounds from the *Aloe* succulent leaves (Fig. 12).

CLASSIFICATION OF *ALOE* SPECIES USING THE ANTHRONE-C-GLYCOSIDES AS BIOCHEMICAL MARKER

The rapid development of natural chemistry has led to the isolation and analysis of a wide variety of secondary metabolites which, in many cases, were found to be of glycoside nature (barbaloin, homonataloin and other C, O - glycosides). The isolation and structural determination of these C, O-glycosides was carried out by means of practical hydrolysis, column chromatography, NMR-, mass-, UV, Vis spectrum, HPLC and optical measurements (Haynes and Henderson, 1960; Franz and Grun, 1983; Yuko *et al*., 1990).

It was found that some *Aloe* species contain anthrone-C-glycosides such as barbaloin, homonataloin and nataloin. These three compounds differ in structure, chromatographic distribution and absorption spectrum (Fig. 1) (Hay and Haynes, 1956; Haynes and Henderson, 1960; Conner *et al*., 1987; Chauser-Volfson and Gutterman, 1998).

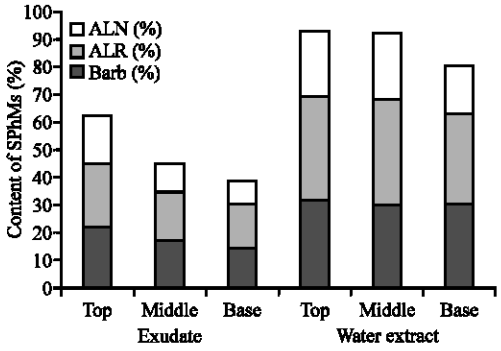

Fig. 12: The content (%) of barbaloin, aloeresin and aloenin in dry weight, in the top, middle or base parts of the succulent leaves of *Aloe arborescense*. The SPhMs were separated from the leaves by two methods: exudates or water extract

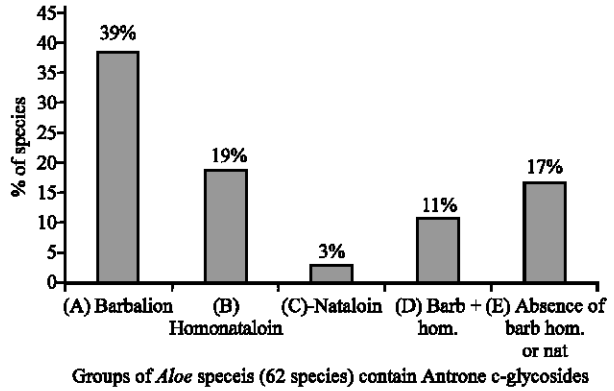

Fig. 13: Groups of Aloe species according to their content of (A) barbaloin, (B) homonataloin, (C) nataloin, (D) barbaloin together with homonataloin and (E) plant species without barbaloin, homonataloin or nataloin. These anthrone-C-glycosides were tested in 62 species originated from South Africa and introduced to the Negev of Israel (Chauser-Volfson and Gutterman 2006)

The results of tests on *A. arborescens*, *A. hereroensis* and *A. mutabilis* are detailed in Chauser-Volfson and Guttermnan (1996, 1997, 1998).

These anthrone-C -glycosides were tested in 62 species originated from South Africa and introduced to the Negev of Israel.

It was found that 39% of these *Aloe* species contain barbaloin (group A).

Another 19% of the species contain homonataloin (group B). Only 3% contain nataloin (group C). 11% of these plants contain barbaloin together with homonataloin (group D). 27% of these plant species didn't contain any of these three anthrone C-glycosides: barbaloin, homonataloin or nataloin (group E) (Fig. 13).

Group A: includes 24 *Aloe* species and it contains different percentages of barbaloin: from 1.5 to 50% in dry weight exudates.

Group B: includes 12 species and contains from 2.1 to 55% homonataloin, but only *A. thraskii* in this group contained a low amount of metabolites (2.1%). *A. jackonii* contains two diastereoisomers of homonataloin (4 and 8%, respectively).

Group D: includes 7 species and contains barbaloin and homonataloin together.

A. melanacanta, A. cameronii and *A. candelubrum* contain 12.8, 8.9 and 9.9% of barbaloin respectively and 21.2, 4.2 and 1.0% homonataloin. *A. gerstneri, A. khamiesensis* and in *A. africana* hybrid contained 9.6, 13.4 and 8.4% barbaloin, respectively and each of these species also contained two homonataloin diastereo-isomers: H_1= 9.1, 25.3, 12% and H_2= 4.7, 14.6 and 10.4% respectively. These diastereo-isomers were isolated using TLC (Rf (H_1) = 0.30-.0.33; Rf (H_2) = 0.52-0.55; Rf (B) = 0.39-0.42.

Group C: includes 2 species: *A. rupestris* and *A. mitriformis,* they contain 18 and 24.7% of anthrone-C-glycoside (nataloin), respectively.

Group E: includes 17 species, which do not contain anthrone-C-glycosides (SPhMs) such as barbaloin, homonataloin and nataloin (Personal communication of Chauser-Volfson).

Among the collection of *Aloe* at Kew Botanical Gardens, London, England, homonataloin occurs in about 15% of the species (Beaumont *et al*., 1984). Among 62 Aloe species tested at Sede Boker, homonataloin occurs in 19% of Aloe species (Personal communication of Chauser-Volfson) (Fig. 13).

It is interesting to know that *Aloe* species which are close related taxonomically, differ in their SPhMs, therefore these materials could not be used as taxonomic markers.

CONCLUSION

- In different *Aloe* plant species it was found different combinations of SPhMs.
- In some leaves of *Aloe* plant species it was found three or four different SPhMs.
- The different SPhMs were found in different plant tissues in the same leaf.
- Most of the SPhMs content is connected to the leaf vascular bundles.
- The younger the leaf the denser is the vascular bundles and therefore the higher the content of the SPhMs.
- Leaf pruning increase the content of the SPhMs in the leaves. The more times the plant is pruned the higher is the SPhMs content of its leaves, up to even 85% of the leaf dry weight.
- Even pruning of one young leaf at the top part of the branch affect an increase in the leaves below. The closer the leaf bellow the one pruned, the higher the content of its' SPhMs. The leaves oriented at the opposite side of the pruned leaf are also affected by increasing their content of SPhMs.
- It was found that the use of water extract method is the more efficient method in comparison to the exudates method, in order to obtain *Aloe arborescens* powder with the highest SPhM content. This is true when the succulent top, middle and base leaf parts were compared.
- The anthrone C-glycosides barbaloin and homonataloin were found in 50% of *Aloe* plant species. Nataloin appear only in 3% of the species. *Aloe* species which are close related taxonomically differ in their SPhMs, therefore these materials could not be used as taxonomic markers.

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