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Barbaloin content of aloe (*Aloe barbadensis*) leaf exudates as affected by different drying techniques

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ABSTRACT

Aloe (*Aloe barbadensis* Mill.) is commercially cultivated for its transparent leaf gel and leaf exudates. The leaf exudates collected from epidermal layer contain anthraquinone glycosides (aloins) mainly barbaloin (aloin A) and isobarbaloin (aloin B). Aloin A is used as a raw material for the production of diacylrhein, a potent drug prescribed for rheumatoid arthritis. Conventional drying of leaf exudates in open sun causes changes in physicochemical properties and altered aloin A and B composition. Various drying techniques, viz. oven drying, freeze drying, shade drying and open sun drying were employed to evaluate the qualitative and physico-chemical changes in final product of aloe leaf exudates. Freeze drying resulted in high quality dried exudates having maximum aloin A content of 54.16%. The other three drying techniques resulted in lower aloin A content in the final dried product of leaf exudates. Fresh aloe exudates contained lower amount of aloin B (4.65% w/v). Sun drying increased aloin B content to 17.73% (to the extent of 2.27 fold) in the final product compared to freeze drying. Shade drying and sun drying lowered the total aloin content by 13.2% and 8% respectively compared to freeze dried exudates. Freeze drying is the most efficient technique to obtain high quality dried aloe exudates having good textural and physicochemical property. Alternately, shade drying with proper ventilation can be employed to get acceptable final product with marginally lower (8%) total aloin content compared to freeze drying.

Key words: Aloe leaf exudates, Anthraquinone content, Drying method, Phytochemical content

Aloe barbadensis Mill. is an important medicinal plant cultivated for the clear leaf gel (contains glucomannans, amino acids, lipids, sterols and vitamins) and latex or leaf exudates. The latex is pale yellowish liquid which oozes out from the leaf lining or epidermal layer when cut. Active principles present in the leaf exudates of *A. barbadensis* are mainly barbaloin (aloinA) (1,8-Dihydroxy-10-(β-D-glucopyranosyl)-3-(hydroxymethyl)-9(10H)-anthracenone10-β-D-Glucopyranosyl-1,8–dihydroxy–3-(hydroxymethyl)-9(10H)-anthracenone) and its diastereoisomer, isaobarbaloin (aloin B) (Gutterman and Chauser-Volfson 2000). Aloe leaf exudate is used as a laxative drug due to its cathartic action and as a bittering agent in alcoholic beverages (Gutterman and Chauser-Volfson 2008). It is also used against indications as seizures, asthma, colds, ulcers, bleeding, amenorrhea, colitis, depression, diabetes, glaucoma, multiple sclerosis, hemorrhoids, peptic ulcers, varicose veins, bursitis, arthritis, and vision problems (Eshun and He 2004).

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Barbaloin (aloin A) constitutes about 1% of the dry leaf weight in 68 species of Aloe investigated (Groom and Reynolds 1987). The highest concentration of barbaloin was found in exudates from young leaves just below the apex and the level decreased in older leaves towards the base of the plant. It is a raw material for production of diacetylrhein (1, 8-diacyl derivative of rhein) which is used for the treatment of osteoarthritis. Although, aloin content ranges between 18 and 60% of aloe leaf exudates on dry weight basis, the content of aloin is drastically reduced due to improper processing techniques and due to complex biochemical reactions. Loss in active principles due to poor processing directly affects the efficacy of herbal preparations. Poorly prepared aloe exudates receive low acceptance as raw material for industrial production of other therapeutic agents. Drying the plant products is an effective way to preserve their desirable qualities, reduce wastages, decrease storage volume and extend their shelf life. Some phenolic compounds are very sensitive to heating, while some are relatively stable when processed at high temperature (Larrauri *et al.* 1997). Drying can be performed by traditional sun drying, microwave drying or oven drying. Drying tends to inactivate the enzymes like polyphenol oxidases which oxidize the phenolic compounds. However, when the drying of plant tissues or extracts is slow,

enzymatic and/or non-enzymatic processes that may occur during drying, may lead to significant changes in the composition of phytochemicals (Capecka *et al.* 2005) which reduce the qualities / medicinal properties. Freeze drying is accepted as the best drying technique for plant materials and it preserves the nutrients, color, and bioactive ingredients (Marques *et al.* 2006). Nevertheless, freeze drying is costly compared to convective hot air drying and other methods (Ratti 2001). However, final products obtained from hot air drying are often characterized by low rehydration capacity and inferior color as well as poor product quality. Relatively, high temperatures (80-100^{o} C) applied in hot air drying often results in the loss of valuable constituents during processing (Zhang *et al.* 2002). Owing to the increased interest for these classes of compounds because of their diverse usage, there is a need for understanding the extraction, processing and storage of aloin. Very little information is available on the comparison of the aforementioned drying methods in term of aloin content and exudates quality. The aim of this study was to investigate the effects of drying techniques (sun drying, hot air oven, and freeze drying) on quality and aloin content of aloe leaf exudates.

MATERIALS AND METHODS

Aloe leaf exudates were collected from healthy fully matured leaves of one year old *A. barbadensis* plants raised in the research farm of ICAR-DMAPR, Boriavi, Anand, Gujarat, India (N 22°55', E072°66', MSL – 45m). Leaves were cut from the plants using a sharp knife by making an incision at the base and kept upside down in a plastic tray to collect the fresh exudates from the leaf. The fresh exudates collected from the cut leaves were immediately taken to the laboratory for weighing and dried using following drying technique until a constant weight was achieved:(i) Open pan drying under shade,(ii) Oven drying at 60^{o} C, (iii) Freeze drying (lyophilization) and (iv) Open pan drying directly under sunlight.

The fresh and dried aloe exudates samples prepared from different drying techniques were analyzed by HPLC at 270 nm with a flow rate of 1 ml/min with a mobile phase of acetonitrile:water (25:75) in a Shimadzu HPLC system having RP 18 column. Aloin content in fresh aloe exudates was carried out by dissolving the contents immediately after collection and weighing. Known amount of leaf exudate is dissolved in deionized water and centrifugation at 10 000 rpm for 5 min, of this 20 µl sample was loaded in a HPLC for analysis. The Shimadzu HPLC systems used consisted of LC 10AD VP pump, Rheodyne sample injector, SPD 10A UV-VIS detector along with Aimil Chromatograph data station for data collection and analysis.

Statistical analysis was conducted with Rstudio (Version 0.98.49, Boston, MA, USA). Results were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

The two major constituents of fresh aloe leaf exudates are barbaloin (aloin A) and isobarbaloin (aloin B). The fresh aloe exudates contained higher amount of aloin A (55.04% w/v) and lower aloin B (4.65% w/v). The barbaloin powder (M/s Sigma-Aldrich) was found to have aloin A and aloin B in a ratio of nearly 2:1. The HPLC chromatograms of fresh aloe leaf exudates (1:10 v/v in methanol) and commercially available pure aloin (100 ppm in methanol) were measured at 254nm. 1A and 1B, respectively. The chromatogram of fresh aloe exudates showed various other components as separate peaks which were eluted before the aloins.

Among the drying techniques, freeze drying (lyophilization) resulted in high quality dried exudates with aloin A content of 54.16% (Fig 1 A). The other three drying techniques resulted in lower aloin A content in the final dried product of leaf exudates. Among the drying techniques, sun drying resulted in dark brown to black powder with 39.19% aloin A content. Shade and oven drying resulted in 14.0 and 16.8% reduction respectively in aloin A compared to freeze drying. Due to continuous vacuum during freeze drying process, the biochemical reactions are prevented and all the original constituents of the tissue or plant extracts are retained without any loss. Freeze dried product was pale yellow to yellowish amorphous powder whereas, other drying techniques resulted in dark brown crystalline product due to the exposure of the material to air during drying process.

Sun drying resulted in notable increase in the aloin B content in the final product. Aloin B content increased to

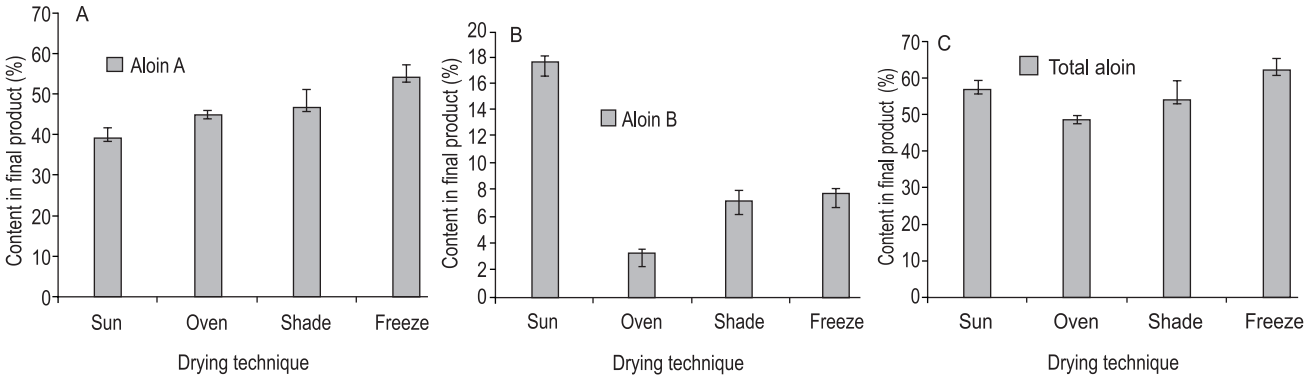

Fig 1 Content of aloin-A (A), aloin-B (B) and total aloin (C) in dried aloe exudates obtained from different drying techniques.

17.73% from 4.65% w/v present in fresh sap. It was nearly 2.27 fold higher as compared to freeze drying. Surprisingly, oven drying resulted in aloin B content of 3.35% and was nearly 57% lower compared to lyophilisation/freeze drying (Fig. 1B). There was a slight decrease (~6.9%) in aloin B due to shade drying compared to freeze drying. Sun drying is also affected by climatic factors, which leads to an uneven loss of phenolic compounds. Some phenolic compounds decompose rapidly under direct sunlight or if dried at elevated temperature (Mueller-Harvey 2001), rosmarinic acid was degraded when it is dried under direct sunlight, and in oven at 60 and 80 ^{o}C (Okuda *et al.* 1989). There was a substantial reduction in aloin A content when the exudates was sun dried which is the most commonly used method for producing aloe exudates/crystals.

Total aloin content of fresh aloe exudate was at 59.68% w/v. Among the drying techniques used, maximum total aloins recovery was obtained from freeze drying with final content of 61.97% w/w. Oven drying resulted in the lowest total aloin content (48.4% w/w) which was nearly 22% lower compared to freeze drying. Shade drying and sun drying resulted in final exudate which was 13.2 and 8% lower total aloin content respectively compared to freeze drying.

Several studies have reported the loss of bioactive principles following high temperature drying methods (Chan *et al.* 2009, Zhang *et al.* 2004, Jayaraman and Gupta 1995, Drouzas *et al.* 1999). Decline in total phenolics and antioxidant content are often accompanied by loss of other bioactive principles (Roy *et al.* 2007). Intense and/or prolonged hot drying may cause significant loss of natural antioxidants, as most of these compounds are relatively unstable at high temperatures as high as 60^{o} C. Yet in some cases, processing causes little or no change to the content and activity of naturally occurring antioxidants, such as carotenoids as some of them (e.g. lycopene) were found to be highly heat stable even after intense or prolonged heat treatment (Nicoli *et al.* 1999). Loss of aloin after sun drying may be caused by enzymatic processes that occurred during sun drying because sun drying did not immediately deactivate degradative enzymes such as polyphenol oxidases; therefore, they are able to degrade or produce complex phenolic compounds before the plant materials are completely dry. The two isomers aloin, A and B, which differ by the position of the glucose group at the anthrone base, their proportions being susceptible to vary depending on the origin of the plants and on the extraction methods used.

Oven heating at over 50^{o}C was shown to rapidly inactivate polyphenol oxidases present in plant materials; however, some of their initial activities might have occurred before actual drying taken place and caused some polyphenols to be degraded. In the present stydy, oven drying resulted in lower aloin B content possible due to inactivation of enzymes and inhibition of steriomeric conversion from aloin A to aloin B in addition to drastic reduction in total aloin content. Solar radiation may also cause some degradation of compounds. The drying techniques employed in the present study, had significantly altered the composition and content isomers of aloin in the dried aloe exudates. The high quality dried exudates with a pale yellow powder having aloin A content of 54.16% in freeze dried product over the fresh leaf exudates may be attributed to the total loss of volatile constituents present in the fresh exudates which concentrated the contents. This technique would have resulted in the efficient removal of volatile materials due to the reduced pressure during the entire drying process.

Sun drying is a slow process and hence the interaction of phenolic compounds in the complex matrix of aloe leaf exudates resulted in inferior quality of final product, possibly due to oxidation of phenolic compounds and steriomeric conversion of aloin A to aloin B by enzymatic reactions. The substantial increase in aloin B in sun drying method compared to other methods, is due to light mediated inter conversion of aloin A to B. Rhein and various 1,8-diacyl derivatives, among which diacerein, are known for their therapeutic properties in the treatment of degenerative disorders of articulations and/or of the connective tissue, such as osteoporosis and rheumatoid arthritis, and in long-term treatment of osteo-arthritis (Malterud *et al*.1993). Industrial production of rhein and diacerein involves acetylation of aloin, followed by oxidation with chromic anhydride (Di Napoli 2002) and hence purity of aloin is essential to obtain higher yield from manufacturing process.

Dried aloe exudates containing maximum barbaloin is preferred as a raw material. Our results show that freeze drying is an efficient technique highly suited for drying of leaf exudate without the loss of aloin and to obtain an appealing pale amorphous powder from aloe. When freeze drying facility is not available, shade drying with good ventilation can be employed to get an acceptable final product with a marginal loss in total aloin content. Oven drying is not recommended due to the lowest total aloin content and poor quality of final product.

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