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RESEARCH PAPER

Effect of age of the leaf and method of gel preparation on antioxidants and microbial count of aloe gel

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SUMMARY :

An experiment was conducted at College of Horticulture, Rajendranagar, Hyderabad during 2009 to study the effect of age of the leaf and method of gel preparation on antioxidants and microbial count of Aloe gel. The experiment was carried out in Completely Randomized Block Design with factorial concept with aloe leaves of four different ages and four methods of gel preparation, replicated thrice with three leaves per replication. Four age groups comprised 8 months, 10 months,12 months and 14 months aged leaves. Similarly four methods of gel preparations were used comprising aloe leaf with skin with filtering, aloe leaf with skin without filtering, aloe leaf without skin with filtering and aloe leaf without skin without filtering which consisted of, total of 16 treatments. The results showed that among four age groups of aloe leaves, 14 months aged leaves recorded higher antioxidant activity (65.73% inhibition of peroxidation and 429.33% Thiobarbituric acid reactive substances) than the rest of the ages of leaves. Among the methods of gel preparation highest antioxidant activity was recorded with skin with filtering (48.35% inhibition of peroxidation and 453.42% Thiobarbituric acid reactive substances) than rest of the methods. Regarding the microbial count in Aloe gel microbial count was noticed in case of 8 months aged leaf recording less bacterial and yeast / mould count (4 and 3 cfu/ml). Higher microbial count was recorded with aloe gel obtained from 14 months aged leaf (12 and 6 cfu/ml). The lowest microbial count was noticed in case of method with skin and with filtering and highest microbial count with gel obtained through the method without skin and without filtering.

KEY WORDS : Antioxidant, Aloe gel , Microbial count

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Aloe can be used as a potential source to develop a wide variety of food products. It can also be incorporated in other food products to enhance their nutritional value like refreshing juice, ready to serve drink, sport drink, soft drink, diet drink, laxative drink, sharbats etc. The fleshy portion can also be converted into candies, squash, jam, bar, munch etc. Additionally, it can be incorporated to dairy products eg. yoghurt, curd, lassi, ice creams as a dairy alternatives. The gel can be dried using suitable drying techniques and the dried powder can be used in the development of various products. Aloe gel is also used in pharmaceutical industry for preparation of ointments, gel preparation, production of tablets and capsules (Hamman, 2008). Antioxidants are a class compounds which prevent certain types of chemical damage caused by an excess of free radicals, charged molecules that are generated by variety of sources. Antioxidants destroy the free radicals which may help to fight cancer, heart diseases and strokes etc. In the present study, Antioxidant activity of aloe gel was measured in the form of per cent TBARS and per cent inhibition of

peroxidation.

EXPERIMENTAL METHODS

The experiment was carried out in Completely Randomized Block Design (CRD) with factorial concept. Experiment was planned with leaves of four different ages and four methods of gel preparation, replicated thrice with three leaves per replication. Four Leaf ages comprised of T_1 - Aloe leaf of 8 months age, T_2 - Aloe leaf of 10 months age, T_3-Aloe leaf of 12 months age and T_4-Aloe leaf of 14 months age. Four Methods of gel preparation consisted of G_1-Aloe leaf with skin with filtering, G_2-Aloe leaf with skin without filtering, G_3-Aloe leaf without skin with filtering and G_4-Aloe leaf without skin without filtering. The experiment consisted a total number of 16 treatments.

Extraction of gel:

Aloe leaves collected were processed within two to three hours after harvest. Leaves of different age groups were collected as per the requirement of the experimental treatments. After harvesting, Aloe leaves are washed thoroughly with clean water for imposing treatments without skin. The aloetic sap was separated from the leaves by cutting the leaves transversely at the base and kept the cut portion touching the ground and allowed the leaf to stand in slanting position for half an hour. Thus, it helps for easy removal of yellow coloured sap. The leaves were again washed thoroughly and the upper thorny tips, side two margins and lower epidermis layer were removed with the help of a sharp knife. A transverse cut was given to the leaf and the gel was scooped out by using knife. The extracted gel was thoroughly homogenized with a blending machine for 15 minutes. Half of this homogenized gel was filtered using a strainer. Thus gel prepared without filtration and the other with filtration were used for experimental purpose.

For the treatments where aloe leaves with skin to be used were thoroughly washed with clean water. The aloetic sap was separated from the leaves by cutting the leaves transversely at the base and kept the cut portion touching the ground and allowed the leaf to stand in slanting position for half an hour. Thus, it helps for easy removal of yellow coloured sap. The leaves were again washed thoroughly and the thorns on the sides of the leaves were removed with the help of a sharp knife and a transverse cut was given to the leaf and the entire leaf along with skin is cut into small pieces and was thoroughly homogenized with a blending machine for 15 minutes. Half of this prepared gel was filtered using a strainer. Both the prepared gels, one without filtration and the other with filtration were used for estimation of antioxidants and microbial count (after storage).

EXPERIMENTAL FINDINGS AND ANALYSIS

The experimental findings obtained from the present study have been discussed in the following heads:

Antioxidants :

The results showed that aloe gel is having antioxidant activity. Antioxidants significantly differed among the different age groups of leaves and method of gel preparation. The antioxidant activity expressed in form of per cent inhibition of per oxidation was presented in Table 1.

Out of four different age groups of leaves, highest antioxidants were recorded with 14 months aged leaves (65.73%) which was significantly superior to the rest of the treatments and was followed by 12 months aged leaf (45.01%). The lowest antioxidants were recorded with 8 months aged leaves (38.17%).

Among the different methods of gel preparation, significantly highest antioxidants (48.35%) were recorded with the treatment (G_1) gel obtained with skin and with filtering method followed by the treatment (G_4) gel prepared without skin without filtering (48.14%). The lowest antioxidants (46.44%) were recorded with the gel prepared without skin and with filtering.

Table 1 : Effect of leaf age and method of gel preparation on inhibition of peroxidation (%) of aloe gel

Leaf age	Method of gel preparation				
	Aloe leaf with skin with filtering (G_1)	Aloe leaf with skin without filtering (G_2)	Aloe leaf without skin with filtering (G_3)	Aloe leaf without skin without filtering (G_4)	Mean
T_1 (8 months)	38.00	38.90	38.40	37.40	38.17
T_2 (10 months)	40.60	40.53	42.36	43.06	41.64
T_3 (12 months)	45.10	46.63	44.50	43.80	45.01
T_4 (14 months)	69.73	64.36	60.50	68.30	65.73
Mean	48.35	47.61	46.44	48.14	
		Leaf age (T)	Method of gel preparation (G)		Interaction (TxG)
S.E.(m)±		0.03	0.03		0.06
C.D. (P=0.05)		0.09	0.09		0.19

Table 2 : Effect of leaf age and method of gel preparation on thiobarbituric acid reactive substances (%TBARS) of aloe gel

Leaf age	Method of gel preparation				
	Aloe leaf with skin with filtering (G_1)	Aloe leaf with skin without filtering (G_2)	Aloe leaf without skin with filtering (G_3)	Aloe leaf without skin without filtering (G_4)	Mean
T_1 (8 months)	532.33	566.00	587.00	555.00	560.08
T_2 (10 months)	460.00	489.00	520.00	477.00	486.50
T_3 (12 months)	420.00	440.00	457.33	437.33	438.66
T_4 (14 months)	401.33	435.00	460.00	421.00	429.33
Mean	453.42	482.50	506.08	472.58	
		Leaf age (T)	Method of gel preparation (G)	Interaction (TxG)	
S.E.(m)±		2.08	2.08	4.16	
C.D. (P=0.05)		6.1	6.1	12.01	

The interaction between age groups of leaves and method of gel preparation was significant on antioxidants. Significantly highest inhibition of peroxidation (69.73%) was recorded with the treatment combination 14 months aged leaves and method of gel preparation with skin and with filtration (T_4G_1) followed by 14 months aged leaves and method of gel preparation with skin and with filtering T_4G_4 (68.30%). The lowest per cent inhibition was recorded with T_1G_4 gel obtained from 8 months aged leaves and method of gel preparation without skin and without filtering (37.40%).

Aloe gel was measured in the form of per cent Thiobarbituric acid reactive substances (Table 2) and there is an inverse relationship between the per cent TBARS and antioxidant activity (Amruta Pritam and Purushottam Kale, 2007). The minimum per cent of TBARS was recorded with 14 months aged leaf (429.33%) followed by 12 months aged leaf (438.66%). The maximum per cent of TBARS was recorded with 8 months aged leaf (560.08%). The highest antioxidant activity was noticed with 14 months aged leaf followed by 12 months aged leaf and lowest antioxidant activity was recorded with 8 months aged leaf. Measurement of bioactivity such as antioxidant capacity becomes more useful for assessing the healthiness of foods than measurement of specific micronutrients (Van Beckel and Jongen, 1997) All the Aloe extracts showed significant antioxidant activity. Hence growth stage plays a vital role in the composition of antioxidant constituents and antioxidant activity of Aloe vera (Hu Yun Xu Juan QiuHui, 2003). Among the methods of gel preparation lowest per cent TBARS (453.42%) was noticed with skin and with filtering and highest was recorded without skin with filtering (506.08%).

Regarding the interaction between age groups of leaves and method of gel preparation was significant on per cent TBARS. Aloe 14 months aged leaf with skin with filtering

Table 3 : Effect of leaf age and method of gel preparation on microbial count of aloe gel

Treatments	Microbial count	
	B	y/m
T_1- Leaf matured for 8 months + with skin with filtering	4	3
T_2- Leaf matured for 8 months + with skin without filtering	5	3
T_3- Leaf matured for 8 months + without skin with filtering	5	3
T_4- Leaf matured for 8 months + without skin without filtering	5	4
T_5- Leaf matured for 10 months + with skin with filtering	6	3
T_6- Leaf matured for 10 months + with skin without filtering	7	4
T_7- Leaf matured for 10 months + without skin with filtering	5	5
T_8- Leaf matured for 10 months + without skin without filtering	6	6
T_9- Leaf matured for 12 months + with skin with filtering	6	3
T_{10}- Leaf matured for 12 months + with skin without filtering	6	3
T_{11}- Leaf matured for 12 months + without skin with filtering	4	4
T_{12}- Leaf matured for 12 months + without skin without filtering	5	4
T_{13}- Leaf matured for 14 months + with skin with filtering	9	6
T_{14}- Leaf matured for 14 months + with skin without filtering	12	6
T_{15}- Leaf matured for 14 months + without skin with filtering	5	4
T_{16}- Leaf matured for 14 months + without skin without filtering	6	5

recorded lowest per cent TBARS(401.33%) followed by 12 months leaf with skin with filtering (420.00%). There is an inverse relationship between per cent TBARS and antioxidant activity. The highest antioxidant activity was recorded with 14 months aged leaf with skin with filtering.

Microbial count :

The results obtained for microbial count in case of gel obtained from different age groups of leaves and by different methods of gel preparation are presented in Table 3. There were significant differences in the microbial count of aloe gel due to different age groups of leaves and method of gel preparation.

Aloe gel and leaf itself has an antimicrobial activity. Highest microbial count (9, 12, 5 and 6 bacterial count, 6, 6, 4 and 5 yeast/mould count at different gel preparation methods) was noticed in case of 14 months aged leaves compared to minimum aged leaves (12, 10 and 8 months aged leaves respectively). From the results, gel obtained from 8 months aged leaf recorded less bacterial and yeast/mould count. Higher amount of microbial count was noticed with aloe gel obtained from 14 months aged leaf is due to the fact that it has higher amount of substrate for the microbes to grow. Similar observation was made by Ismet (2008) where aloe extract of 1 year aged leaf showed no sensitivity but 2 and 3 years aged leaves showed significant sensitivity to microbes. The comparative antimicrobial activities of the gel and leaf of *Aloe vera* were tested against certain inoculum's strains and the results tend to support to the popular use of both Aloe vera gel and leaf (Agarry *et al.*, 2005).

Significantly highest bacterial and yeast/mould count was noticed with treatment T_{14} (12 and 6 cfu/ml, respectively) where gel obtained from 14 months aged leaves by the method of gel preparation with skin without filtering followed by treatment T_{13} (9 and 6 cfu/ml, respectively) gel obtained from 14 months aged leaves by the method of gel preparation with skin with filtering. The lowest bacterial and yeast/mould count was noticed with the treatment T_1 (4 and 3 cfu/ml, respectively) where the gel obtained from 8 months aged and method of gel preparation with skin with filtering.

It can be inferred from the results obtained that 14 months aged leaf having higher antioxidant activity and more of microbial count when compared to the remaining aged leaves. The method of gel preparation with skin with filtering was found to be the best method for gel extraction in *Aloe vera*.

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