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Efficiency of Asiatic pennywort (*Centella asiatica* L. Urban) extract in the stabilization of *Aloe vera* gel

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Abstract
An experiment was conducted in the Dept. of Plantation Crops and Spices, College of Agriculture, Vellayani, Kerala Agricultural University, during 2016 -17 for the development of a protocol for the stabilization of fresh aloe gel using *Centella asiatica* extracts. Fresh aloe gel samples were treated with three forms (decoction, aqueous and alcoholic extracts) of *Centella asiatica* in order to find out their efficiency in the stabilization of aloe gel. Observations on pH, conductivity, T.S.S, vitamin C and microbial load of samples were recorded at weekly intervals for a period of one month and compared with fresh aloe juice and also with aloe juice treated with a chemical preservative (sodium benzoate) as control. 5ml alcoholic extract treated samples recorded a pH, conductivity, TSS and vitamin C content which was almost equal to that of pure gel up to two weeks of storage. Assessment of microbial load of these samples after two weeks of storage revealed no bacterial, fungal, actinomycetes and *E.Coli* colonies in the samples. Hence it can be concluded that *Aloe vera* juice sample treated with 5ml alcoholic extract of *Centella asiatica* could retain the characteristics of fresh juice up to two weeks of storage and was free from microbial contaminants.

Keywords: *Centella asiatica*, *Aloe vera*, stabilization, aloe juice

Introduction
In recent years, *Aloe vera* has assumed an important role in formulation of natural products both in food and cosmetic industry. Gel has been traditionally employed for many beneficial properties for human health. Its gel the parenchymatic tissue of aloe leaves, contains over 98-99% water and more than 60% of the dry matter is made up of polysaccharides (Segovia *et al*., 2009) [1]. Due to its therapeutic and functional properties and beneficial effects on humans, the use of *Aloe vera* in the formulation of food products has increased (Miranda *et al*., 2009) [2]. In view of its highly perishable nature, mainly from the microbiological point of view, aloe has to be processed in order to extend its shelf life. Just after harvesting, decomposition of the fresh gel starts, due to the action of enzymes and of bacteria normally present in the leaves, resulting in loss of biological activity. It must be processed as soon as possible after cutting, through a process called "stabilization", in order to preserve its components and to ensure quality of gel. This degradation can be prevented by drying the gel or by adding suitable preservative and antioxidants or an addition of algal sulphated polysaccharide (Yaron, 1993) [3]. The gel stabilization process in *Aloe vera* included admixing with a heated gel an antioxidant and adjusting the gel pH to a range of 3-3.5 followed by cooling (Rezaee *et al*., 2003) [4]. The stabilizing antioxidants may be a tocotrienol/tocopherol blend, rosemarinic acid, polyphenols or any combination thereof. Parameters that are routinely used in the evaluation and identification of commercial *Aloe vera* gels are pH, malic acid, and conductivity (Ni and Tizard, 2004) [5]. The International Aloe Science Council (IASC) has presented guidelines for levels of these parameters in *Aloe vera* gels (Waller *et al*., 2004) [6]. pH and conductivity are the two criteria commonly used for judging the freshness of aloe gel (Ni and Tizard, 2004) [5]. Fresh aloe gel is reported to possess a pH nearing 4.7 and conductivity 2.4 mS (Waller *et al*., 2004) [6]. Reports regarding the synergistic effects of four species of plants belonging to Liliaceae, Zingiberaceae, Theaceae and Punicaceae on stabilization of aloe gel and their potential as natural antiseptics and oxidation resistant materials are available (Jian Guo *et al*., 2004) [7]. In the present study, the potential of *Centella asiatica* (Family: Apiaceae), a medicinal herb with reported antioxidant potential, in the stabilization of aloe gel is explored.

Materials and Methods

The experiment was conducted in the Dept. of Plantation Crops and Spices, College of Agriculture, Vellayani, Kerala Agricultural University, during 2016 -17 for the development of a protocol for the stabilization of fresh aloe gel using *Centella asiatica* extracts. Fresh aloe gel samples were treated with three forms (decoction, aqueous and alcoholic extracts) (Fig.1) of *Centella asiatica* extracts (Fig 1). Aqueous extract was prepared by grinding 10g of fresh herb with 50ml of distilled water using mortar and pestle followed by filtration. Hot water extract was prepared by boiling 10g of fresh herb in 50ml of water followed by filtration while alcoholic extract was prepared by grinding 10g of sample with 50ml of 80 % ethanol followed by filtration.

For standardizing the dosage, 20 ml of liquidized and filtered *Aloe vera* gel was treated with graded volumes (1.0ml, 2.0ml, 3.0ml, 4.0ml and 5.0ml) of each form of extract with three replications and kept at ambient temperature. Observations on pH, conductivity, T.S.S, vitamin C and microbial load of each sample were recorded at weekly intervals for a period of one month and compared with fresh aloe juice and also with aloe juice treated with a chemical preservative (sodium benzoate) as control. pH was measured using pH meter (Digital pH meter MK VI) and conductivity using Elico Digital Conductivity meter (Model CM 180). For measuring T.S.S Hanna Refractometer (Model HI 96801) was used while Vit C content was estimated following the procedure recommended by Sadasivam and Manickam, 2008 [8].

Results and Discussion

Variation in pH in *Centella asiatica* treated samples

Observations on the pH of treated samples during the storage period are given in Table1. On the first day of observation no significant difference in pH was noticed among the samples and all values agreed with the values prescribed by IASC (4.7 – 4.9). One week after storage slight increase in pH was observed in all samples except those treated with alcoholic extracts and chemical preservative (T11 to T16). T14 and T15 could maintain this value during the entire storage period while in T16 a slight increase was noticed during the 4thweek. A possible explanation for the increase of pH in aloe gel was given by Lodi and Rossin, 1995 [9]. Malic acid is an excellent indicator of gel freshness. This acid is produced naturally in aloe leaves during Crassulacean Acid Metabolism (CAM). Lactic acid formation is associated with ageing of aloe gel, resulting in a concentration decline of malic acid levels. Under poor handling/storage conditions, in the presence of bacteria, malic acid can be broken down to form lactic acid which might have resulted in the increase in pH of stored gel. Thus lactic acid occurrence is an indication of gel decay (O'Brien *et al*., 2011) [10].

Variation in conductivity in *Centella asiatica* treated samples

In the case of conductivity, also similar trend was noticed. On the first day of observation no significant difference in conductivity among the samples were noticed and all maintained a stable value (2.8 – 2.9). One week after storage a slight increase in conductivity was observed in all samples except those treated with 5 ml alcoholic extracts and chemical preservative (T15 & T16).The 5ml alcoholic extract and control maintained a conductivity of 2.8 throughout the observational period. The fresh juice shows wide variation from initial value 2.8 to 3.4. (Table 2). A possible explanation for this relationship is that glucose can also be converted into lactic acid which can result in an increase free ions or conductivity within decaying aloe gels. Levels of conductivity in gels appear to be species specific (O'Brien *et al*., 2011) [10]. Maintenance of a steady conductivity in T15 indicates that addition of *Centella asiatica* alcoholic extract could prevent gel decay during storage at room temperature.

Variation in TSS and Vitamin C concentration in *Centella asiatica* treated samples

After one week of preliminary observation on pH and conductivity, the TSS and vit C content of promising treatments with agreeable visual quality and odour were analysed. These included 5ml hot water extract (T5), 5ml aqueous extract (T10), 1ml, 3ml and 5 ml alcoholic extracts (T11,T13,T15) treated samples, fresh juice (T16) and that with chemical preservative (T17) .

After one week of observation the treatments showed wide variation in TSS (6.6 – 20.4) and T16 recorded the highest value (20.4) which was on par with T15. A general reduction in TSS was noticed during storage, and during the 2nd week also T16 recorded the highest value. However T15 also recorded a comparable value (17.0) with fresh gel. In the case of control a drastic reduction in TSS was noticed throughout the storage period. (Table 3). As above said explanation during gel decay conversion of malic acid and glucose (free ions) into lactic acid increases in pH values (as lactic acid is a weaker acid than malic acid) and conductivity, a decrease in sugar concentration was also occurred in decayed samples (Tungala *et al*., 2011) [11].

In the case of Vit C also wide variation was noticed among treatments (29.78 – 44.65). T15 recorded the highest value (44.65) which was on par with T16 (42.50). A general reduction in Vit C content was noticed during storage, however during the 2nd week T15 and T16 recorded the highest values (40.39) which were comparable with that of fresh gel. In the case of control a drastic reduction in Vit C was noticed throughout the storage period (Table 4). Oxygen is the most destructive ingredient in juice causing degradation of vitamin C (Padayatty *et al*., 2003) [12]. The retention of vitamin C is often used as an estimate for the overall nutrient retention of food products because it is by far the least stable nutrient; it is highly sensitive to oxidation and leaching into water soluble media during storage (Franke *et al*., 2004) [13]. It begins to degrade immediately after harvest and degrades steadily during prolonged storage (Murcia *et al*., 2000) [14].

Microbial load in *Centella asiatica* treated samples

Assessment of microbial load of the samples revealed no bacterial, fungal, actinomycetes and E.Coli colonies in the samples treated with alcoholic extract (5.0ml) of *Centella asiatica* during these period (Fig. 2). Reports indicate that naturally occurring alkaloids and their synthetic derivatives in *Centella asiatica* have bactericidal activities (Zainol *et al*., 2003) [15]. Many plant flavonoids have antimicrobial activity (Cushnie and Lamb, 2005) [16] and these Flavanoids are known for substantial antimicrobial activity in *Centella asiatica* (Alcaraz *et al*., 2000) [17]. Thus it was explained that high concentration of alcoholic extract of *Centella asiatica* have antimicrobial property.

Conclusion

To conclude *Aloe vera* juice sample treated with 5ml alcoholic extract of *Centella asiatica* could retain the characteristics of fresh juice up to two weeks of storage and was free from microbial contaminants.

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Table 1: Variation in pH in *Centella asiatica* treated samples at weekly intervals

Treatments	0th day	7thday	14thday	21stday	28thday
T1 - 20ml gel + 1 ml h.w.e.	4.6	4.7	3.9	4.1	4.2
T2 - 20ml gel + 2 ml h.w.e.	4.6	4.8	4.0	4.1	4.2
T3 - 20ml gel + 3 ml h.w. e.	4.6	4.8	4.0	4.1	4.2
T4 -20ml gel + 4 ml h.w.e.	4.6	4.8	4.0	4.2	4.3
T5 -20ml gel + 5 ml h.w.e.	4.6	4.8	4.0	4.2	4.3
T6 - 20ml gel + 1 ml aq.e.	4.6	4.7	3.9	4.9	3.9
T7 - 20ml gel + 2 ml aq.e.	4.7	4.8	3.9	4.9	4.0
T8 - 20ml gel + 3 ml aq.e.	4.7	4.8	3.9	5.0	4.0
T9 - 20ml gel + 4 ml aq.e.	4.7	4.8	3.9	4.0	4.1
T10 - 20ml gel + 5 ml aq.e.	4.7	4.8	3.9	4.2	4.2
T11 - 20ml gel + 1 ml al.e.	4.6	4.7	4.6	4.6	4.6
T12 - 20ml gel + 2 ml al. e.	4.7	4.7	4.6	4.6	4.7
T13 - 20ml gel + 3 ml al.e.	4.7	4.7	4.6	4.6	4.7
T14 - 20ml gel + 4 ml al.e.	4.7	4.7	4.7	4.7	4.7
T15 - 20ml gel + 5 ml al.e.	4.7	4.7	4.7	4.7	4.7
T16 - 20ml gel + c.p.	4.7	4.7	4.7	4.7	4.8
T17 - control	4.7	4.6	4.9	5.0	5.8
CD	0.01	0.01	0.01	0.01	0.01
SEM(+-)	0.005	0.005	0.005	0.005	0.005

h. w. e – hot water extract aq. e. - aqueous extract al. e. – alcoholic extract c. p. – chemical preservative

Table 2: Variation in conductivity in *Centella asiatica* treated samples at weekly intervals (mS)

Treatments	0th day	7thday	14thday	21stday	28thday
T1 - 20ml gel + 1 ml h.w.e.	2.9	3.2	3.3	3.3	3.4
T2 - 20ml gel + 2 ml h.w.e.	2.8	3.2	3.2	3.2	3.3
T3 - 20ml gel + 3 ml h.w. e.	2.8	3.1	3.1	3.2	3.2
T4 -20ml gel + 4 ml h.w.e.	2.8	3.1	3.1	3.2	3.2
T5 -20ml gel + 5 ml h.w.e.	2.8	3.0	3.1	3.1	3.1
T6 - 20ml gel + 1 ml aq.e.	2.9	3.2	3.2	3.3	3.3
T7 - 20ml gel + 2 ml aq.e.	2.9	3.1	3.2	3.2	3.3
T8 - 20ml gel + 3 ml aq.e.	2.8	3.1	3.1	3.2	3.2
T9 - 20ml gel + 4 ml aq.e.	2.8	3.1	3.1	3.1	3.2
T10 - 20ml gel + 5 ml aq.e.	2.8	2.9	3.0	3.1	3.2
T11 - 20ml gel + 1 ml al.e.	2.8	3.0	3.0	3.1	3.2
T12 - 20ml gel + 2 ml al. e.	2.8	2.9	3.0	3.1	3.1
T13 - 20ml gel + 3 ml al.e.	2.8	2.9	3.0	3.0	3.1
T14 - 20ml gel + 4 ml al.e.	2.8	2.8	2.9	3.0	3.1
T15 - 20ml gel + 5 ml al.e.	2.8	2.8	2.8	2.8	2.8
T16 - 20ml gel + c.p.	2.8	2.8	2.9	2.8	2.8
T17 - control	2.8	3.0	3.2	3.3	3.4
CD	0.01	0.01	0.01	0.01	0.01
SEM(+-)	0.005	0.005	0.005	0.005	0.005

h. w. e – hot water extract aq. e. - aqueous extract al. e. – alcoholic extract c. p. – chemical preservative

Table 3: Variation in TSS in *Centella asiatica* treated samples at weekly intervals (mg/dL)

Treatments	7thday	14thday	21stday	28thday
T5 -20 ml gel + 5 ml h.w.e.	8.2	7.0	6.4	5.4
T10 - 20ml gel + 5 ml aq.e.	6.6	5.9	5.1	4.2
T11 - 20ml gel + 1 ml al.e.	14.9	13.4	11.0	8.6
T13 - 20ml gel + 3 ml al.e.	17.0	14.4	12.2	10.0
T15 - 20ml gel + 5 ml al.e.	18.2	17.0	14.6	12.2
T16 - 20ml gel + c.p.	20.4	18.2	16.6	12.6
T17 - control	14.4	13.7	12.1	10.2
CD	1.2	0.7	1.2	1.0
SEM(+-)	0.71	0.41	0.71	0.59

h. w. e – hot water extract aq. e. - aqueous extract al. e. – alcoholic extract c. p. – chemical preservative

Table 4: Variation in Vit C in *Centella asiatica* treated samples at weekly intervals (mg/dl)

Treatments	7thday	14thday	21stday	28thday
T5 -20 ml gel + 5 ml h.w.e.	36.16	31.91	25.53	23.12
T10 - 20ml gel + 5 ml aq.e.	29.78	27.65	23.12	20.72
T11 - 20ml gel + 1 ml al.e.	31.87	29.78	27.65	25.53
T13 - 20ml gel + 3 ml al.e.	38.29	36.16	31.95	29.78
T15 - 20ml gel + 5 ml al.e.	44.65	40.39	38.29	36.16
T16 - 20ml gel + c.p.	42.50	40.39	38.29	36.16
T17 - control	36.16	34.04	31.91	26.91
CD	5.41	5.98	5.50	6.07
SEM(+-)	3.22	3.55	3.27	3.61

h. w. e – hot water extract aq. e. - aqueous extract al. e. – alcoholic extract c. p. – chemical preservative

Fig 1: *Centella asiatica* decoction, aqueous and alcoholic extracts treated samples

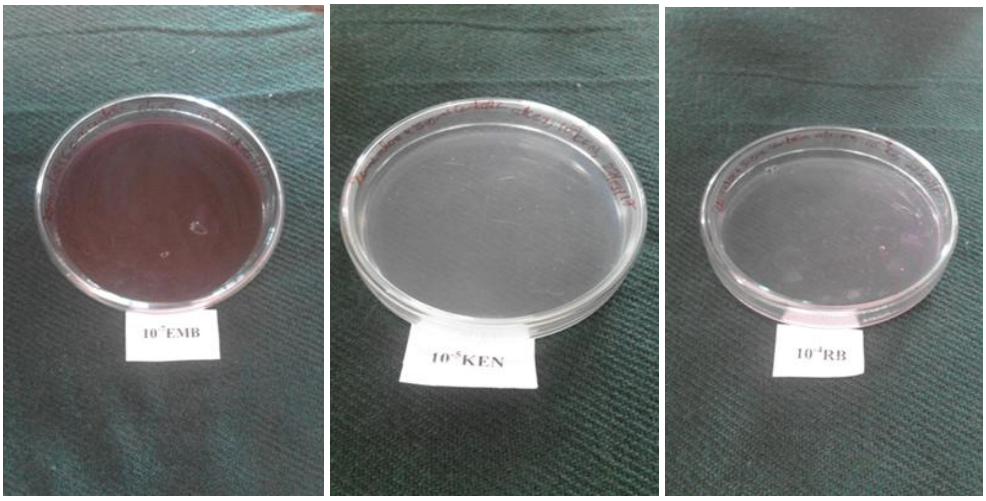

Fig 2: Microbial analysis of 5 ml alcoholic extract treated samples of *Centella asiatica*.

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