Original Article

# Qualitative assessment of *Aloe vera* as natural tissue fixative: An institutional study

Anjali Narwal[1], Esha Singh[1], Jigyasa Duhan[2], S. K. Dhattarwal[3], Virender Singh[4], Mala Kamboj[1], Anju Devi[1], Shruti Gupta[5]

Departments of [1]Oral Pathology, [2]Conservative and Endodontics, [4]Oral Surgery and [5]Oral Anatomy, Post Graduate Institute of Dental Sciences, [3]Department of Forensic Medicine, Post Graduate Institute of Medical Sciences, Rohtak, Haryana, India

## Abstract

**Objectives:** The art of microscopy is only appreciable in well-fixed tissues. We conducted this study to determine the effectiveness of *Aloe vera* as a tissue fixative and compare its results with the natural fixatives already studied in the literature.

**Materials and Methods:** A pilot study was tried out using commercially available fresh chicken and fish with *Aloe vera,* and then after getting the promising results similar study protocol was carried out using 10-autopsied human tissue. Four natural fixatives-30% jaggery solution, 20% honey solution, 20% sugar solution, 20% *Aloe vera* solution and 10% formalin were used for fixation in the study. Fixation of tissues was carried out at room temperature for 24 h. All pre- and postfixation measurements were recorded using stereomicroscope and its software. The difference between pre-and postfixation was calculated and later, all pieces were kept for routine tissue processing followed by routine staining. The tissue sections were assessed for quality, and the whole procedure was blinded among three oral pathologists who scored them.

**Results:** The mean percentage of shrinkage in each bit with different reagents was calculated. The shrinkage seen with 10% formalin and 20% *Aloe vera* were more likely similar. Among all the natural fixatives, qualitatively also *Aloe vera* excelled and its results were comparable to that of formalin.

**Conclusion:** The use of *Aloe vera* in the present study as fixative is the first of its kind, as exhaustive search of the literature shows only its use as transport media in dentistry.

**Keywords:** *Aloe vera*, fixative, formaldehyde

**Address for correspondence:** Dr. Anjali Narwal, Department of Oral Pathology, Post Graduate Institute of Dental Sciences, Rohtak - 124 001, Haryana, India.
E-mail: anjalinarwal@yahoo.com

**Submitted:** 01-Dec-2020, **Accepted:** 30-Nov-2021, **Published:** 21-Mar-2023

## INTRODUCTION

The proper processing of tissue as soon as it is removed from the biopsy site will form the foundation of all good microscopic preparations. Immediately, after removal, the tissue should be put into suitable fixative solution that will fix all the cells of the tissue in a "life-like state." If the tissue is unfixed or dried out, then the necessary details of the cell and its components will be lost and it will be difficult for the pathologist to make a proper diagnosis.[1] An ideal fixative should possess the following characteristics: (1) It should fix the tissue in the life-like state by preventing autolysis and bacterial contamination/putrefaction; (2) It should not change the volume or shape of tissue; (3) It should prevent shrinkage, osmotic damage and swelling; (4) It

Access this article online

Quick Response Code:

Website: www.jomfp.in

DOI: 10.4103/jomfp.jomfp_489_20



**How to cite this article:** Narwal A, Singh E, Duhan J, Dhattarwal SK, Singh V, Kamboj M, *et al*. Qualitative assessment of *Aloe vera* as natural tissue fixative: An institutional study. J Oral Maxillofac Pathol 2023;27:33-8.

should harden the tissue, allowing easy tissue sectioning and should convert semifluid consistency of cells to an irreversible semisolid consistency; (5) It should allow clear staining of sections; (6) It should allow optimal optical differentiation.[2,3]

"Formalin is the universal and well-established fixative used in routine histopathology" that possesses all the characteristics of an ideal fixative. The major benefit stems from its regular and worldwide use for more than 100 years and all the scientific knowledge gathered on it.[1] It is easily available, cost-effective, allows long-term storage, preserves lipids well and fairly convenient to use and store. It is acknowledged as the closest thing, which is available as the perfect fixative with no clear "all-purpose" substitute found to date.[4]

In contrast, formaldehyde has been classified by the International Agency for Research on Cancer as a potential cancer-causing agent, which can cause nasopharyngeal cancer in humans.[5] A study by Lu *et al*. provides strong evidence which corroborates that both the cytotoxicity and genotoxicity contribute to the carcinogenesis of inhaled formaldehyde in respiratory nasal epithelium.[6] Second, the chemical action of formalin binds severely to DNA, RNA and proteins, which makes them difficult or impossible to extract them for any molecular studies.[7] Since then, a variety of chemicals have been tried to compete with the standards set by formalin. Literature reported that bee honey as fixative preserves morphology of the tissue as done by formalin.[8] As jaggery and sugar have the same composition as that of honey, so they have also been tried and tested for fixation by various researchers.[9] While searching the literature for natural substitute of formalin, we came across *Aloe vera*, which possesses many benefits. *Aloe vera* extract has been used in healthy food and beverages. It has been used topically to heal the wound and has been tried as a laxative. It is also found in skin products such as cosmetics and sunblocks due to its antioxidant properties. It acts as immune booster, antiviral, anti-inflammatory agent as well as possesses antifungal properties.[10-12]

The primary aim of fixation is to preserve the tissues, prevent bacterial putrefaction, autolysis and increase the refractive index of the tissue.[13] With this aim, a study was conducted to compare the tissue fixation capability of jaggery, honey, sugar solution and *Aloe vera* with that of formalin and qualitatively compare their results microscopically using routine processing and staining. There is always tissue shrinkage once the tissue is immersed in the fixative overnight. Ideally, it should be minimum to enhance the viewing of the maximum tissue removed by the surgeon. Hence, this criterion of minimal shrinkage by ideal fixative was also evaluated in this study using stereomicroscope and its software.

## MATERIALS AND METHODS

The present study was conducted after obtaining ethical permission from the institutional ethics committee vide letter no. PGIDS/IEC/2016/45 dated 07.10.2016.

### Preparation of fixatives

Fixative I (30% jaggery solution) was prepared using 30 mg of commercially available jaggery, which was broken down into small pieces and then hand mixed with 100 ml of distilled water. Fixative II (20% processed honey) was prepared by mixing 20 ml of pure processed honey (commercially available, pH-4.6) with 100 ml of distilled water. For Fixative III (20% sugar solution), 20 mg of cane sugar (commercially available) was mixed with 100 ml of distilled water. For Fixative IV, the external surface of *Aloe vera* leaf was washed and disinfected with 70% ethanol. The gelatinous transparent substance was cut off from the external shield and triturated to get a homogeneous gel. For 20%, 20 mg of gel was mixed with 100 ml of distilled water. Fixative V that was 10% formalin was prepared by mixing 100 ml of formaldehyde solution in 900 ml of distilled water. All the fixative solutions were freshly prepared and stirred well to get homogeneous solutions. The pH of all fixative solutions at the used concentrations ranged from 4.5 to 5.7. Over the period, molds are likely to grow on all these natural substances; hence, thymol crystals were added to inhibit microbial and fungal activity.

### Procedure

In this study, before using the autopsy tissue, the study protocol was tested with commercially available fresh chicken leg as well as commercially available fish. Each tissue was cut into five pieces of approximately equal size and a suture was tied on its one end to confirm its orientation under stereomicroscope. Each bit was measured along its length (X-axis) and width (Y-axis) with the help of stereomicroscope (Radical Steromicroscope-9) and its software (DGI size-50.1). All prefixation measurements were recorded and each bit was placed into five different containers containing (I) 30% Jaggery solution, (II) 20% Honey solution, (III) 20% Sugar solution, (IV) 20% *Aloe vera* solution and (V) 10% formalin. Formalin was taken as positive control and distilled water as negative control. Tissues were fixed at room temperature for 24 h and after that, tissues were removed from the fixatives

and postfixation measurements were noted again. The tissue was placed in the same manner as it was put during prefixation with the help of suture. This suture helped in the proper orientation of tissue under stereomicroscope. The difference between pre- and postfixation measurements of each bit in each fixative was recorded [Figure 1].[14] After these measurements, the tissues were kept for routine tissue processing followed by routine hematoxylin and eosin (H and E) staining. Since chicken tissue showed only muscles microscopically, so similar protocol was repeated with commercially available fish. After getting satisfactory results with them, 10 autopsy samples of skin from 10 different deceased bodies within 3 h of death by the Department of Forensic Medicine were procured and similarly, they were also cut into five pieces each and prefixation measurements were taken. After 24 h of fixation in each fixative, postfixation measurements were noted. Fifty tissues, i.e., 5 pieces of each 10 autopsy samples fixed in five types of fixatives were qualitatively assessed under microscope after routine processing and staining. The criteria for qualitative comparison of tissue sections used were (a) staining quality, (b) cellular outline, (c) nuclear details, (d) absence of tissue foldings and (f) overall morphology under light microscopy. Each criterion was scored with the range of 0–2, poor being 0 and 2 being good by three oral pathologists who scored the results of the stained slides and the whole procedure was blinded. Individual score for each criterion in each fixative was recorded and the mean was calculated. The Kruskal–Wallis test was used for statistical analysis.

The difference in the measurements, i.e., pre-and postfixation along X-axis (length) and Y-axis (width) were tabulated, and the mean percent shrinkage for each fixative was calculated. Normality test was applied to the results. Since the data were nonparametric so the Mann–Whitney test was used for statistical analysis. Qualitative and quantitative results for each sample were compared among various fixative groups used in this study.

## RESULTS

All natural fixatives used in this study were able to preserve the tissue morphology during fixation. Best results in fixation, both qualitative and quantitative were obtained in 10% formalin followed by *Aloe vera*, sugar solution, honey and then jaggery. Tissue shrinkage in formalin was almost similar to that of *Aloe vera*. More shrinkage was seen in sugar solution in comparison to *Aloe vera* and formalin. Jaggery showed maximum shrinkage along both X-and Y-axis [Table 1]. Tissues after fixation were processed under routine protocol and stained with hematoxylin and eosin. Formalin-fixed tissue sections after processing showed the best nuclear and cytoplasmic details under microscope. The second best results were seen with *Aloe vera* followed by sugar solution and then honey and jaggery [Table 2]. *Aloe vera* showed good nuclear and cytoplasmic staining as well as good overall morphology with no tissue foldings [Figure 2]. Results of staining with sugar solution [Figure 3] were also good and better than that of honey [Figure 4] and jaggery [Figure 5]. Honey and jaggery also displayed satisfactory staining in nucleus and cytoplasm but overall morphology of tissues was poor and exhibited abundant foldings.

**Table 1: Tissue shrinkage seen in different fixatives**

| Reagents | X% (SD) | Y% (SD) |
|---|---|---|
| 20% *Aloe vera* | 2.57 (3.37) | 1.61 (0.81) |
| 10% formalin | 2.43 (1.61) | 2.07 (0.89) |
| 20% honey | 2.12 (3.2) | 0.93 (0.38) |
| 30% jaggery | 2.64 (1.18) | 3.7 (2.7) |
| 20% sugar solution | 2.38 (1.2) | 2.12 (2.6) |

SD: Standard deviation

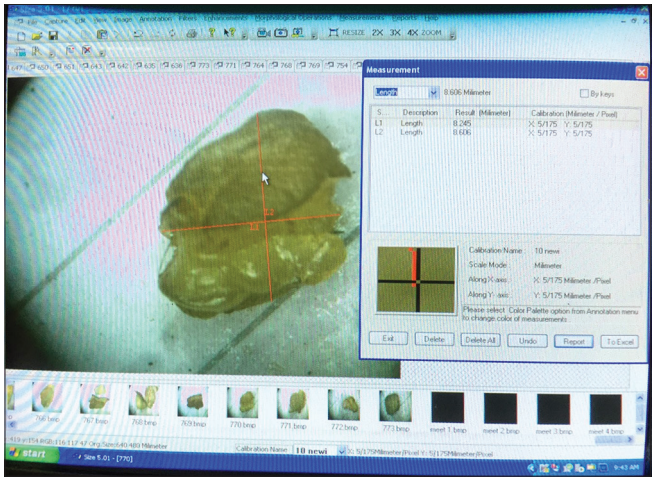


**Figure 1:** Steremicroscope software (DGI size-50.1) window showing orientation as well as the shrinkage of tissue along X-axis and Y-axis

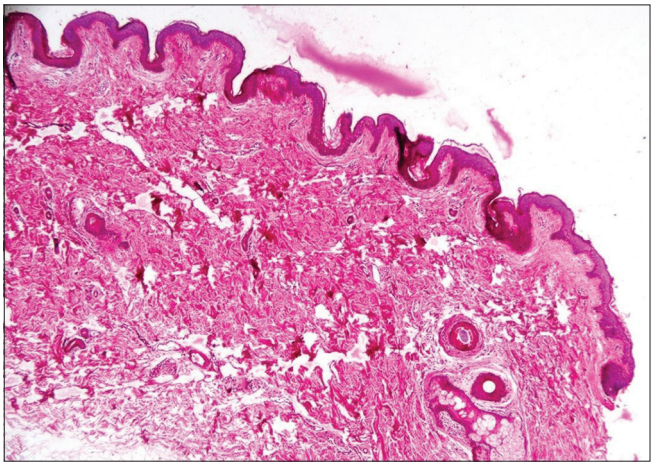

**Figure 2:** *Aloe vera* fixed tissue sections showing good cellular details and minimal tissue folding under microscope (H&E × 4)

**Table 2: Mean qualitative score of individual characteristics for each fixative solution**

| Characteristic | Mean (SD) | | | | | *P* |
|---|---|---|---|---|---|---|
| | 20% sugar solution | 20% *Aloe vera* | 10% formalin | 30% jaggery | 20% honey | |
| Staining quality | 1.50 (0.707) | 1.80 (0.422) | 2.00 (0.000) | 1.30 (0.823) | 1.10 (0.876) | 0.029 |
| Cellular quality | 1.10 (0.876) | 1.90 (0.316) | 2.00 (0.000) | 0.30 (0.483) | 0.80 (0.789) | 0.000 |
| Nuclear detail | 1.20 (0.789) | 2.00 (0.000) | 2.00 (0.000) | 0.80 (0.789) | 0.80 (0.789) | 0.000 |
| Absence of tissue folding | 1.70 (0.675) | 1.80 (0.632) | 1.90 (0.316) | 1.00 (0.816) | 1.40 (0.843) | 0.022 |
| Overall morphology | 1.60 (0.699) | 1.70 (0.675) | 2.00 (0.000) | 0.90 (0.876) | 1.10 (0.876) | 0.007 |

SD: Standard deviation

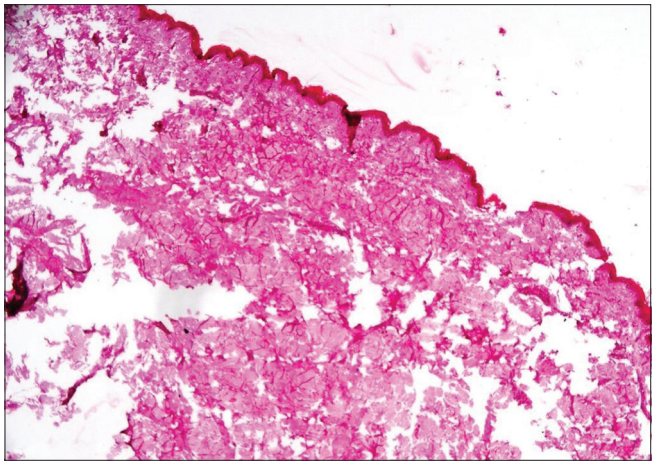

**Figure 3:** Sugar solution fixed tissue sections under microscope (H&E × 4)

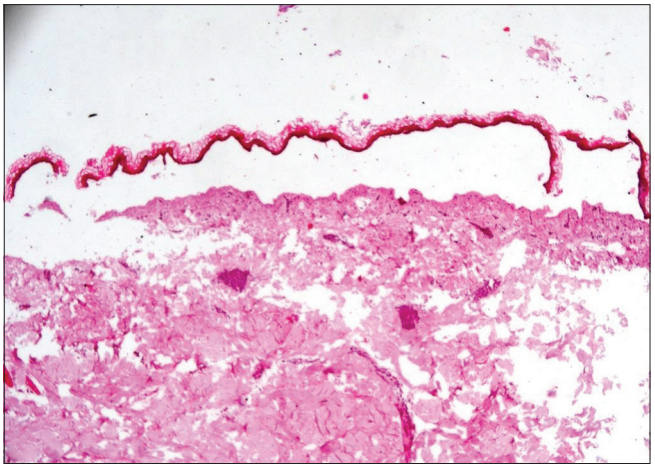

**Figure 4:** Tissue sections fixed with honey under microscope (H&E ×4)

## DISCUSSION

Ideally, it is the fixative, which decides the fate of the tissue section. If the biopsied tissue is not fixed properly, its poor fixation will be reflected in its architecture and cytoplasmic details. Neutral buffered formalin is a well-known fixative and widely used in histopathological laboratories for routine histopathology and immunohistochemistry. Numerous studies have been conducted insurge of an ideal fixative but to date, there is not even a single fixative that can substitute formalin. As it is hazardous in nature and potential carcinogen that can cause nasopharyngeal cancer, several attempts have been made in the past also to find a natural compound which can replace it for tissue fixation.

"For centuries, honey has been shown to be a successful antibacterial agent having the potential to preserve compounds without any harmful effects on users." Al Maaini and Bryant found that tissue fixed using honey at low concentrations at room temperature give results that are as good as seen in formalin-fixed control tissues.[15] Antioxidative and antibacterial effects of honey could be attributed to its properties such as low pH, high osmolarity and the presence of components, i.e., phenol inhibine and hydrogen peroxide.[9] After this, many studies have confirmed that honey could be used as a safe substitute to formalin for conventional and immunohistochemical staining methods.[16,17] Jaggery and sugar, derivatives of sugarcane juice, are well known for their preservative properties. Harish Nayaka *et al*. in their study, confirmed the antioxidant and cytoprotective activity of jaggery.[18] Patil *et al*. conducted a study using honey, jaggery and sugar solution as fixative and this was the first attempt to do so as no existing literature shows the use of jaggery and sugar as formalin substitutes. They compared the fixative property of all these natural substances, as they do not require additional equipment, are nonhazardous and compatible with routine processing and staining. It was suggested that these natural substances can be used where formalin may not be available at the time of biopsy and as well as on large scales such as screening camps.[9]

The use of *Aloe vera* gel extracts in health foods, beverages and moisturizing cosmetics is well known. Historically, *Aloe vera* has been used topically to heal wounds and for various skin conditions and orally as a laxative.[19] *Aloe vera* is a member of liliaceae family. This medicinal plant is green with tapered leaves that are filled with a transparent viscous gel.[20] The inner gel of *Aloe vera* contains more than 75 active ingredients. About 98%–99% of gel is made up of water and the remaining 1%–2% contains active compounds including aloemannan, naphthoquinones, aloesin, acemannan, aloin, aloe emodin, aloeride, methylchromone, saponin, amino acids, flavonoids, sterols and vitamins.[21] Glucomannan, mannose derivative,

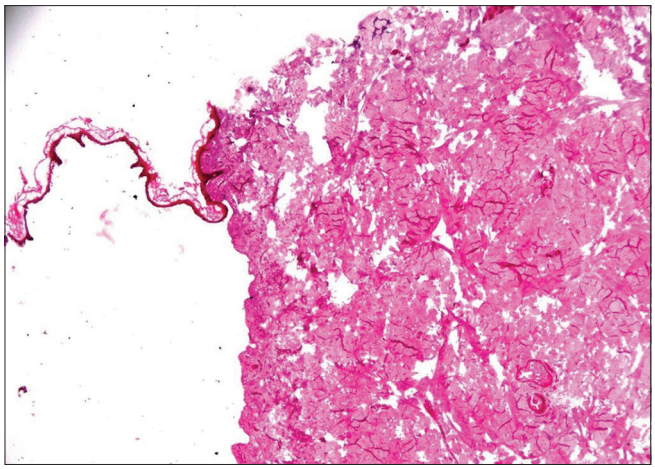

**Figure 5:** Tissue sections fixed with jaggery under microscope (H&E ×4)

cellulose, hemicelluloses and acetylated compounds are the major polysaccharides found in *Aloe vera* gel, which possess linear chains of glucose and mannose molecules.

The majority of biological activities of *Aloe vera* can be attributed to the polysaccharides present in it, which have a synergistic action with other components of *Aloe vera*.[22] The pharmacological actions of *Aloe vera* gel include antioxidant, immune-boosting, antibacterial, anti-inflammatory and hypoglycemic properties.[21,23]

A number of studies have been reported emphasizing *Aloe vera*'s role in dentistry for various purposes such as:

1. Being a good antiseptic and anti-inflammatory, it is used in the treatment of gingivitis and periodontitis
2. Owing to its antiviral and antifungal properties, it is used in the treatment of oral herpes and candidiasis in denture wearers
3. The absence of abrasive content in *Aloe vera* tooth gel makes it a good substitute for normal dentifrices in sensitive teeth
4. *Aloe vera* is a viscous gel that acts as a lubricant during biomechanical preparation in root canal treatment[24]
5. *Aloe vera* extract acts as a good transport and storage media for avulsed tooth as it preserves the periodontal ligament (PDL) cell viability. Badakhsh *et al*. recommended *Aloe vera* as suitable storage media for avulsed teeth.[25] Literature search revealed another study which stated that periodontal fibers near the cementum of the avulsed tooth stored in Aloe vera were intact and thick.[26] Chantarawaratit *et al*. observed the effect of acemannan (potential component of *Aloe Vera*) on periodontal regeneration and found that it significantly increased PDL cell proliferation, vascular endothelial growth factor, Type I collagen and alkaline phosphatase activity. The potential of *Aloe vera* toward successful replantation can be attributed to the periodontal cell proliferating potential of its active components such as acemannan.[27] The high success rate of *Aloe vera* in protecting the cell viability when used as storage media might be attributed to its antibacterial and antifungal properties
6. The role of *Aloe vera* in tissue engineering could be attributed to the synergistic mode of action of many bioactive compounds present in it. They can infiltrate into tissues and increase the transport and activities of biological factors involved in tissue engineering, thus promoting cell migration, proliferation and growth.[22]

To the best of our knowledge, the present study appears to be the first attempt in dentistry, where *Aloe vera* has been used as tissue fixative and not as storage or transport media. The proposed mechanism of action of *Aloe vera* as tissue fixative is that the presence of fructose and mannose (aloemannan and acemannan) in inner clear gel of its leaf when exposed to adequate pH, i.e., acidic converts these components into aldehyde and they further behave like routine formaldehyde in fixation. *Aloe vera* gel having good infiltrative property makes the tissue fixed uniformly and evenly. Other qualitative properties of *Aloe vera* such as antibacterial, antifungal make it a good choice for fixing the tissue by preventing its putrefaction and decomposition.

## CONCLUSION

Natural fixative is the arena, which requires further exploration and more such studies need to be conducted in future to establish *Aloe vera* as a potential substitute. Since the qualitative results of *Aloe vera* with routine H and E staining have been promising so even areas of histochemical and immunohistochemical markers can be tried with *Aloe vera* in future times.

### Financial support and sponsorship

Nil.

### Conflicts of interest

There are no conflicts of interest.

## REFERENCES

1. Rosai J. Why microscopy will remain a cornerstone of surgical pathology. Lab Invest 2007;87:403-8.
2. Rai R, Bhardwaj A, Verma S. Tissue fixatives: A review. Int J Pharm Drug Anal 2016;4:183-7.
3. Bancroft JD, Gamble M, editors. Theory and Practice of Histological Techniques. 5th ed. London: Churchill Livingstone; 2002. p. 63.
4. Hopwood D. Fixatives and fixation: A review. Histochem J 1969;1:323-60.
5. IARC Working Group on the Evaluation of Carcinogenic Risks to

Humans. Formaldehyde, 2-butoxyethanol and 1-tert-butoxypropan-2-ol. IARC Monogr Eval Carcinog Risks Hum. 2006;88:1-478.
6. Lu K, Collins LB, Ru H, Bermudez E, Swenberg JA. Distribution of DNA adducts caused by inhaled formaldehyde is consistent with induction of nasal carcinoma but not leukemia. Toxicol Sci 2010;116:441-51.
7. Gillespie JW, Best CJ, Bichsel VE, Cole KA, Greenhut SF, Hewitt SM, *et al*. Evaluation of non-formalin tissue fixation for molecular profiling studies. Am J Pathol 2002;160:449-57.
8. Ozkan N, Salva E, Cakalağaoğlu F, Tüzüner B. Honey as a substitute for formalin? Biotech Histochem 2012;87:148-53.
9. Patil S, Premalatha B, Rao RS, Ganavi B. Revelation in the field of tissue preservation – A preliminary study on natural formalin substitutes. J Int Oral Health 2013;5:31-8.
10. EMA. Community Herbal Monograph on Aloe Barbadensis MILLER and on Aloe (Various Species, Mainly Aloe Ferox MILLER and its Hybrids). London, UK: European Medicines Agency; 2006.
11. Park YI, Jo TH. Perspectives of industrial application of *Aloe vera*. In: Park YI, Lee SK, editors. New Perspectives on Aloe. New York (NY), USA: Springer Science-Business Media; 2006. p. 191-200.
12. Steenkamp V, Stewart MJ. Medicinal applications and toxicological activities of aloe products. Pharm Biol 2007;45:411-20.
13. Sabarinath B, Sivapathasundaram B, Sathyakumar M. Fixative properties of honey in comparison with formalin. J Histotechnol 2014;37:21-5.
14. Kerns MJ, Darst MA, Olsen TG, Fenster M, Hall P, Grevey S. Shrinkage of cutaneous specimens: Formalin or other factors involved? J Cutan Pathol 2008;35:1093-6.
15. Al Maaini R, Bryant P. The effectiveness of honey as a substitute for formalin in the histological fixation of tissue. J Histotechnol 2006;29:173-6.
16. Al Maaini R, Bryant P. Honey as an alternative to formalin in the demonstration of connective tissue components. J Histotechnol 2008;31:67-72.
17. Mandy G, Bryant P. Immunohistochemical evaluation of ductal carcinoma in breast after preservation in honey. J Histotechnol 2009;32:54-9.
18. Harish Nayaka MA, Sathisha UV, Manohar MP, Chandrashekar KB, Dharmesh SM. Cytoprotective and antioxidant activity studies of jaggery sugar. Food Chem 2009;115:113-8.
19. Hajhashemi V, Ghannadi A, Heidari AH. Anti-inflammatory and wound healing activities of Aloe littoralis in rats. Res Pharm Sci 2012;7:73-8.
20. Joseph B, Raj SJ. Pharmacognostic and phytochemicalpeoperties of Aloe veralinn- An overview. Int J Pharm Sci Rev Res 2010;4:106-10.
21. Fani M, Kohanteb J. Inhibitory activity of *Aloe vera* gel on some clinically isolated cariogenic and periodontopathic bacteria. J Oral Sci 2012;54:15-21.
22. Rahman S, Carter P, Bhattarai N. *Aloe vera* for tissue engineering applications. J Funct Biomater 2017;8:E6.
23. Surjushe A, Vasani R, Saple DG. *Aloe vera*: A short review. Indian J Dermatol 2008;53:163-6.
24. Sujatha G, Kumar GS, Muruganandan J, Prasad TS. *Aloe vera* in dentistry. J Clin Diagn Res 2014;8:I01-2.
25. Badakhsh S, Eksandarian T, Esmaeilpour T. The use of *Aloe ver*a extract as a novel storage media for the avulsed tooth. Iran J Med Sci 2014;39:327-32.
26. Jain D, Dasar PL, Nagarajappa S. Natural products as storage media for avulsed tooth. Saudi Endod J 2015;5:107-13.
27. Chantarawaratit P, Sangvanich P, Banlunara W, Soontornvipart K, Thunyakitpisal P. Acemannan sponge stimulate alveolar bone, cementum and periodontal ligament regeneration in a canine class II furcation defect model. J Periodontal Res 2014;49:164-78.