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The UV Spectroscopic and Chromatographic Methods of Hydroalcoholic Extracts of *Aloe vera*

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ABSTRACT

Aloe vera which is also known as Aloe barbadensis Miller,is a plant that is commonly used for medicinal purposes and as treatments for various health issues. It produces two substances; gel and latex, which are used for commercial household products, halal food and cosmetics. Aloe gel is the clear, jelly-like substance found in the inner part of the Aloe leaf while Aloe's yellow latex comes from the peel. Aloe vera is able to provide therapeutic effects such as wound healing, anti-inflammatory, antioxidant, laxative and antimicrobial properties. The objective of this study was to investigate the extracts via spectrophotometry (λ = 200 – 400 nm) and liquid chromatography. After 21 days, the ultraviolet spectra showed the evidence of the water molecules interactions and the hydroxyl groups in hydroalcoholic extracts. Significant peaks were also observed in the chromatograms. Further studies evaluating the stability of A. vera extracts should be carried out.

Keywords: *Aloe; chromatography; extraction; spectroscopy*

1. INTRODUCTION

The name Aloe is derived from the Arabic word "Alloeh", which means "shining and bitter substance" and from the Latin word "vera" that brings the meaning of "true" [1]. The *Aloe* has more than 400 species and one of its species is *A. vera* [2]. The illustration of the plant and the cross section of the leaf is shown in Figure 1. Other *Aloe* species that are commonly used for medicinal and cosmetic purposes include *A. arborescens, A. ferox* and *A. brevifolia* [3].

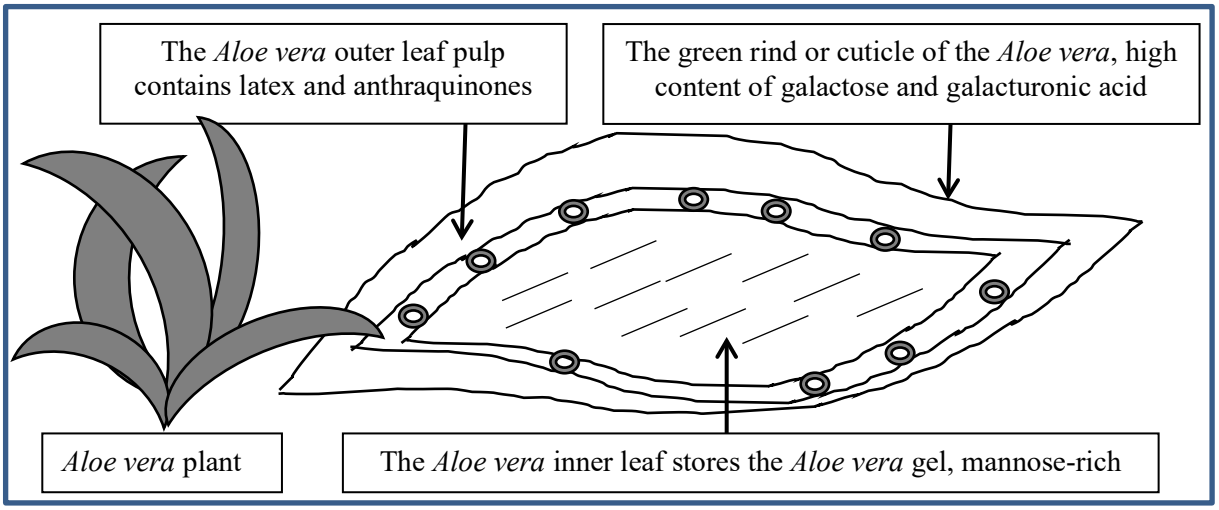

Figure 1: The cross section of an *Aloe* leaf [4-5] (Source: Chandegara *et al*., 2014; NIEHS, 2018).

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Aloe vera resembles the features of a cactus. It is a perennial and succulent plant with green leaves arranged in a pattern of a rose at the stem. The leaves are fleshy and sharp,with a pointed edge that consists of a thick epidermis covered by cuticle surrounding the mesophyll [2]. It originates from the Mediterranean region, but it can be found in subtropical areas [6]. *Aloe* leaf consists of two parts, with each producing different substances that have a completely different composition and therapeutic properties. The inner parenchymal tissues form a clear, thin, tasteless, jelly-like material.

The outer tubules, just beneath the outer green rind or cutinized epidermis of the leaves, produce bitter yellow exudates. The biochemicals and their derivatives are phenolic compounds that include aloin and aloe-emodin (Figure 2). These pharmacologically active anthraquinones from the outer layer of the leaf could function as laxatives [6, 7]. The inner mucilaginous pulp called *Aloe* gel lies in the center of the leaf. This *Aloe* gel consists primarily of water and polysaccharides such as pectins, hemicelluloses, glucomannan, acemannan (Figure 3), and mannose derivatives. It also contains amino acids, lipids, sterols, tannins, and enzymes. In addition, *Aloe vera* contains various vitamins including Vitamin A, C and E that provide antioxidant effects [7].

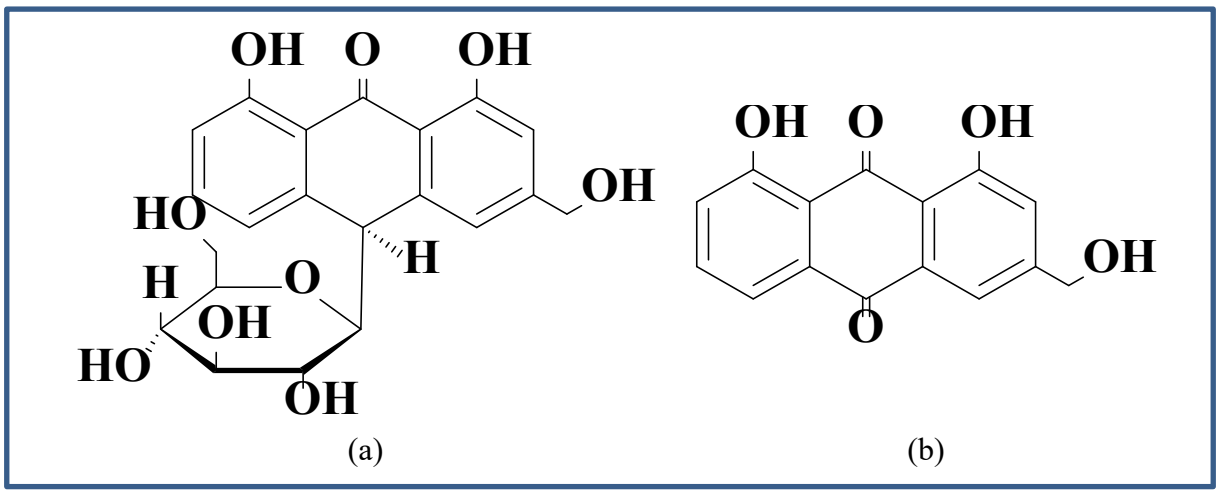

Figure 2: The chemical structures of (a) aloin and (b) aloe-emodin anthraquinones.

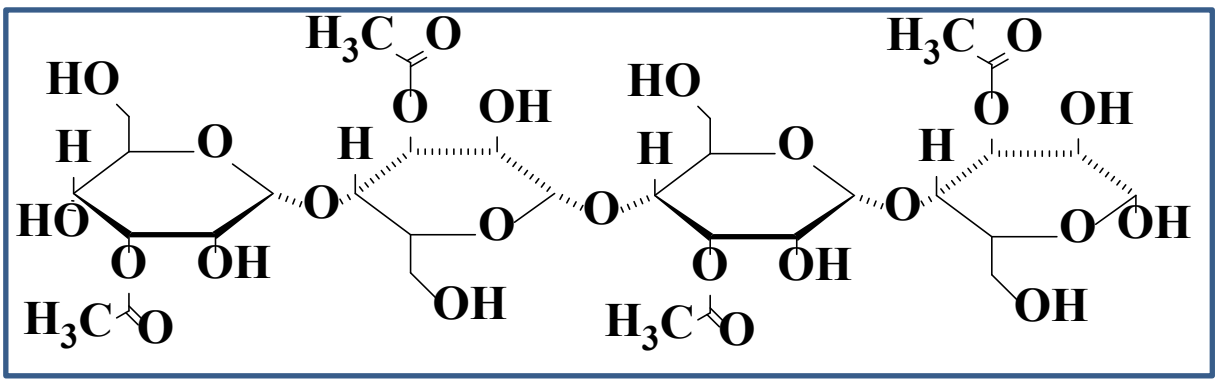

Figure 3: The chemical structure of acemannan (β-(1,4)-acetylated mannan-based polysaccharide).

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2. EXPERIMENTAL

2.1. General

The literature search on *Aloe vera* was conducted electronically (e.g. Science Finder, Medline, Scopus and Google Scholar). The articles were thus analyzed [8]. The laboratory experiments involving the extraction, spectroscopic measurements and reversed-phase high performance liquid chromatography (RP-HPLC) of the leaves were conducted.

2.2. Extraction of the Aloe vera

Organic *Aloe vera* leaves were purchased from the local hypermarket. The green, outer part of the leaves, were removed. The inner gel was scrapped and cut into pieces. It was mixed with methanol and ethanol (Merck) for extraction at room temperature.

2.3. UV spectrophotometry of the Aloe extracts

The Genesys^{TM} 10S dual beam UV/Visible spectrophotometer with spectral bandwidth of 1.8 nm and wavelength accuracy of ± 1.0 nm, was used in this procedure. A pair of 1 cm matched quartz cells was utilized, in order to measure the absorbance of the extracts. The samples were left for 20 days, and the UV spectral data was recorded within the range of 200 to 400 nm [9].

2.4. Reversed-Phase High Performance Liquid Chromatography of the Aloe extracts

One mL of the methanolic extract was transferred into a vial through 0.45 µm polytetrafluoroethylene membrane filter. Next, the vial was placed in the autosampler. The mobile phase consisted of acetonitrile (CH_3CN) and 0.01% formic acid. The RP-HPLC system included a Model 1100 pump supplied with a multi solvent delivery system, an Agilent C18 (5 µm, 4.6 x 250 mm) column and a photodiode array detector [10]. It was set up to run in a gradient elution as follows: 0 min, 10:90; 3 min, 10:90; 30 min, 90:10; 35 min, 90:10; 36 min, 10:90; and 45 min, 10:90. The flow rate was set as 1 mL/min (temperature of the column = 25ºC) and the UV absorbances were measured at λ = 210, 254 and 280 nm. The peaks in the chromatograms were observed, recorded and reviewed. A triplicate trial was made (sample volume : 10.000 µL per injection) and the results were discussed.

3. RESULTS AND DISCUSSION

3.1. Clinical Studies on Aloe Extracts

The extraction of the phytochemicals of *A. vera* could be accomplished by using centrifugation [4] and vigorous shaking [11]. In addition, the thin layer chromatographic profile of *A. vera* extract was also established [10, 12]. The application of *A. vera* was discussed [2, 13] and various characteristics of *A. vera* such as the phytocomponents, pharmacological properties and the clinical aspects of the plant extract were reviewed (Table 1) [14, 6, 15].

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Table 1. *Clinical Studies on Aloe Vera.*

Main points	Source
A review on the properties, mechanism of action and clinical uses of *A. vera*.	[3]
A literature survey on the traditional, phytochemical and pharmacological properties of *A. vera*	[7]
A summary of clinical uses of *A. vera* in dentistry	[6]
A review on the therapeutic properties and applications of *A. vera*	[2]
A list of clinical application of *A. vera* in dentistry	[15]
Focus on the composition and biological properties of *A. vera*	[16]
Observation on contradictory evidence for the use of *A. vera* in cancer radiation	[14]

3.2. The UV spectrophotometry of the Aloe extracts

The extracts resulted maximum absorption at λ = 279 [9] - 300 [17] nm (Figure 4). From the spectra, the water molecules and the hydroxyl group in hydroalcoholic extract resulted UV signals around λ = 220 nm [18]. Two distinct areas were observed, with absorption in UV region for the both extracts, at λ = 270 nm and 290 nm [18]. Therefore, the wavelengths for the RP-HPLC could be selected within the range of λ = 200 - 300 nm, specifically λ = 260-280 nm, for aloin [19-20].

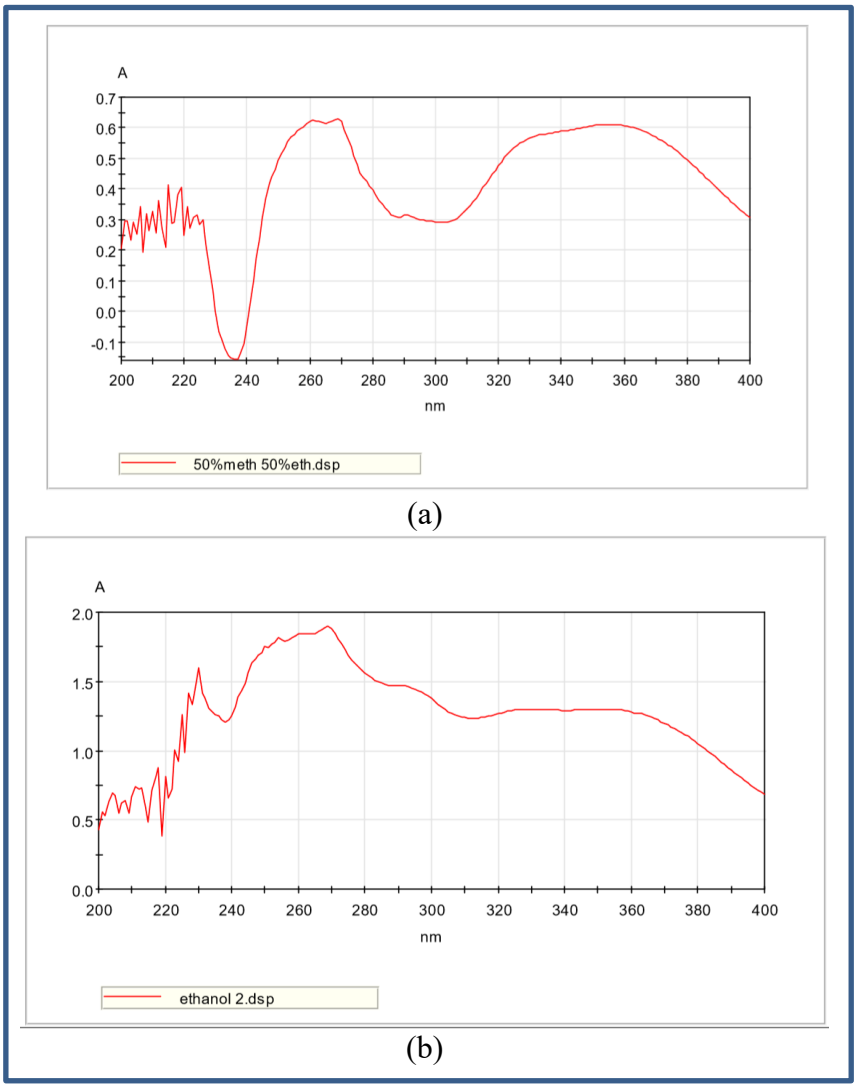

Figure 4: The UV spectral of (a) methanol and (b) methanol/ethanol (50:50) extracts of *Aloe vera*.

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3.3. Reversed-Phase High Performance Liquid Chromatography of the Aloe extracts

From the chromatogram (Figure 5), the combination of mobile phases was a comparatively polar mixture. The compounds which were eluted earliest (retention time, R_T = 1.674 minutes) were more polar, compared to the furthest eluted (R_T = 8.724 minutes). The chromatogram also showed some unresolved, minor peaks, that were not well isolated (R_T = 8.1 – 8.3 minutes). This could be improved by modifying the solvent gradient and/or the injection volume. Therefore, this preliminary result would become a reference for future chromatographic experiments [21-22].

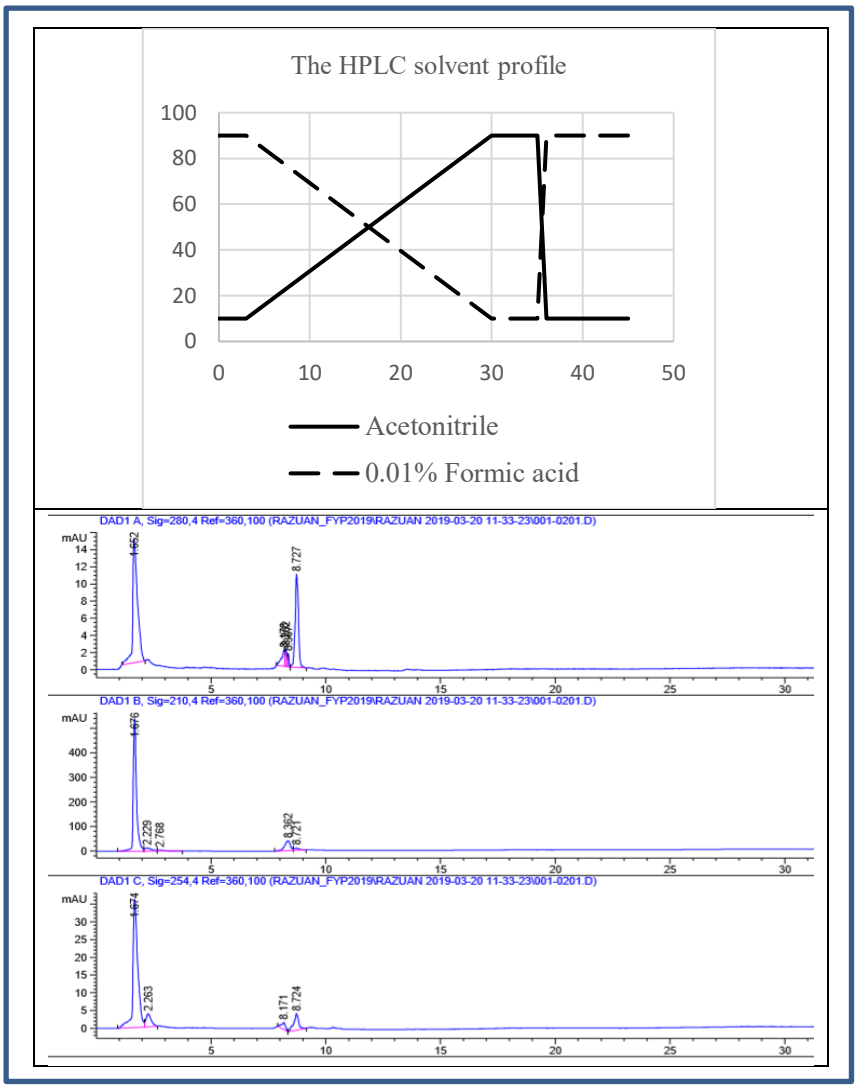

Figure 5: The chromatogram for the *Aloe* extract, recorded at λ = 210, 254 and 280 nm.

It is suggested that aloe emodin could be separated much earlier, at retention time, R_T = 1.674 minutes. Later, the anthrone *C*-glycosides [aloin A (barbaloin) and aloin B (isobarbaloin) could be eluted, respectively at R_T = 8.171 and 8.721 minutes. These *Aloe* compounds could be identified by comparing their retention times with the monograph. Some unresolved, minor peaks, that were not well isolated (R_T = 2.2 and 8.3 minutes) could be attributed to the other polar metabolites of aloins, for example, the aloe emodin anthraquinone (Figure 2) and rhein (Figure 6). The RP-HPLC technique appears to be adequate for routine analysis of the *Aloe*

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extract. Meanwhile, acetylated mannan-based polysaccharide (Figure 3) could be fractionated via high performance gel permeation chromatography (HP-GPC) [23-24].

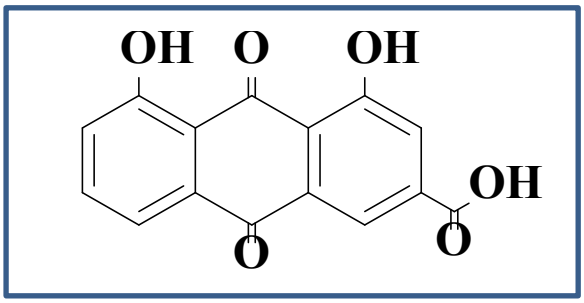

Figure 6: The chemical structures of rhein.

4. CONCLUSION

Aloe vera leaves contain natural compounds in the hydroalcoholic extracts. The compounds are applicable for commercial and pharmaceutical purposes, owing to the presence of complex chemical compositions. There are about seventy potentially active constituents, such as the vitamins, enzymes, minerals, lipids, sugars, lignin, saponins, salicylic acids and amino acids. Researchers have identified acemannan or partially acetylated mannan as the primary polysaccharide of the gel, while others found pectic substance as the primary polysaccharide. Spectrophotometric analysis is recognized as the analytical tool in conforming the molecular interactions of the hydroalcoholic extracts of *Aloe vera.*

Further studies concerning the stability of the *Aloe* extracts using liquid charomatography should be carried out. The RP-HPLC data showed good sensitivity for the detection of compounds. It is concluded that those compounds with the highest absorbance values, were eluted within nine minutes, with the solvent ratio of 30:70 (CH_3CN:H_2O).

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REFERENCES

[1] Metro, D., Cernaro, V., Papa, M., & Benvenga, S. (2018). Marked improvement of thyroid function and autoimmunity by *Aloe barbadensis* Miller juice in patients with subclinical hypothyroidism. *J Clin Transl Endocrinol*, 11, 18–25.
[2] Maan, A. A., Nazir, A., Iqbal Khan M. K., Ahmad, T., Zia R, Murid, M., & Abrar, M. (2018). The therapeutic properties and applications of *Aloe vera*: A review. *J Herb Med*, 12, 1–10.
[3] Surjushe, A., Vasani, R., Saple, D. G. (2008). *Aloe vera*: a short review. *Indian J Dermatol*., 53(4), 163–166.
[4] Chandegara, V. K., & Varshney, A. K. (2014). Effect of Centrifuge Speed on Gel Extraction from *Aloe Vera* Leaves. *J Food Process Technol*., 5(1), 1–6.
[5] National Institute of Environmental Health Sciences (NIEHS), United States of America (2018), accessed via https://www.niehs.nih.gov/health/topics/agents/aloe/index.cfm
[6] Deepthi, K. & Pradeep Kumar, R. (2018). *Aloe vera* - Nature's healer. *J. of Pharm. Res.*, 12(1),

p-ISSN 1675-7939; e-ISSN 2289-4934

1-3.
[7] Sharma, P., Kharkwal, A. C., Kharkwal, H., Abdin, M. Z. & Varma, A. (2014). A Review on Pharmacological Properties of *Aloe vera*. *Int. J. Pharm. Sci. Rev. Res*, 31-37.
[8] Boland, A., Gemma Cherry, M., & Dickson, R. (2014). Doing a Systematic Review: A Student's Guide, SAGE
[9] Maitra, J. & Kalpana. (2017). Extraction, Identification and Antibacterial Activity of Aloin Extracted From *Aloe Vera*. *Eur. J. of Biomedical and Pharmaceutical Sciences*, 4(8), 945-948.
[10] Mandrioli, R., Mercolini, L., Ferranti, A., Fanali, S. & Raggi, M. D. (2011). Determination of aloe emodin in *Aloe vera* extracts and commercial formulations by HPLC with tandem UV absorption and fluorescence detection. *Food Chem*., 126, 387-393.
[11] Agrawal, P., Kotagiri, D., Kolluru, V. C. (2018). Comparative Analysis of Antimicrobial Activity of Herbal Extracts against Pathogenic Microbes. *Adv Biochem Biotechnol.,* ABIO-163.
[12] Daburkar, M., Lohar, V., Rathore, A. S., Bhutaha, P., & Tangadpaliwar, S. (2014). An in vivo and in vitro investigation of the effect of *Aloe vera* gel ethanolic extract using animal model with diabetic foot ulcer. *J Pharm Bioall. Sci*., 6, 205-212.
[13] Rahman, S., Carter, P., & Bhattarai, N. (2017). Aloe Vera for Tissue Engineering Applications. *J. Funct. Biomater*., 8(1), 6.
[14] Farrugia, C.-J. E., Burke, E. S., Haley, M. E., Bedi, K. T., & Gandhi, M. A. (2019). The use of aloe vera in cancer radiation: An updated comprehensive review, *Complementary Therapies in Clinical Practice*, 35, 126-130.
[15] Rathod, S. R., Raj, A., Sarda, T. & Maske, S. (2018). *Aloe vera*: A natural remedy. *SRM Journal of Research in Dental Sciences*, 9, 32-36.
[16] Singh, B., Mohan, R., Maurya, A., & Mishra, G. (2018). Phytoconstituents and biological consequences of *Aloe vera*: A focused review. *Asian J. of Pharm. and Pharmacol.*, 4(1), 17-22.
[17] Muñoz, O. M., Leal, X., Quitral, V., Cardemil, L. (2015). Extraction, Characterization and Properties of the Gel of *Aloe Vera* (*Aloe barbadensis* Miller) Cultivated in Chile. *Med Aromat Plants*, 4, 199.
[18] Carac, A., Boscencu, R., Patriche, S., Dinica, R. M., Carac, G., & Gird, C. E. (2016). Antioxidant and Antimicrobial Potential of Extracts from *Aloe vera* leaves. *Rev. Chim. (Bucharest)*, 67(4), 654 – 658.
[19] Ravi, S., Kabilar, P., Velmurugan, S., Ashok Kumar R., & Gayathir, M. (2011). Spectroscopy Studies on the Status of Aloin in Aloe vera and Commercial Samples. *J. of Exp. Sci.*, 2(8), 10-13.
[20] Tarkase, K. N., & Danve, A. V. (2015). Development and Validation of Spectrophotometric Method for Simultaneous Estimation of Aloin and Sennoside in Suppository Dosage Form. *Int. J. Pharm. Sci. Rev. Res*., 31(2), 195-199.
[21] Azaroual, L., Liazid, A., Barbero, G. F., Brigui, J., Palma, M. & Barroso, C. G. (2012). Improved Chromatographic Methods for Determination of Bioactive Compounds from *Aloe vera* Leaves, ISRN Chromatography, vol. 2012, Article ID 609095, 7 pages. https://doi.org/10.5402/2012/609095.
[22] Sánchez-Machado, D. I., López-Cervantes, J., Mariscal-Domínguez, M. F., Cruz-Flores, P., Campas-Baypoli, O. N., Cantú-Soto, E. U., & Sanches-Silva, A. (2017). An HPLC Procedure for the Quantification of Aloin in Latex and Gel from *Aloe barbadensis* Leaves, *Journal of Chromatographic Science*, 55 (3), 251–257.
[23] Shi, X.-D., Nie, S.-P., Yin, J.-Y., Que, Z.-Q., Zhang, L.-J., & Huang, X.-J. (2017). Polysaccharide from leaf skin of *Aloe barbadensis* Miller: Part I. Extraction, fractionation, physicochemical properties and structural characterization. *Food Hydrocolloids*, 73, 176-183.
[24] Shi, X.-D., Yin, J.-Y., Zhang, L.-J., Li, O.-Y., Huang, X.-J. & Nie, S.-P. (2019). Studies on polysaccharides from leaf skin of *Aloe barbadensis* Miller: Part II. Structural characteristics and molecular properties of two lower molecular weight fractions. *Food Hydrocolloids*, 86, 50-61.

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