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EVALUATION OF QUALITY OF ALOE VERA WITH CEDAR WOOD OIL AS IMMERSION MEDIUM FOR MICROSCOPY IN PERIPHERAL SMEAR

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ABSTRACT

Background: Aloe Vera gel mainly has antifungal, anti inflammatory property due to which it is in major demand in the herbal area of research. Aloe Vera is clear gel with refractive index of 1.3; hence it was tried as substitute to cedar wood oil. On prolonged use cedar wood oil damages the objective lens.

Aim: To evaluate the quality of Aloe vera as immersion medium for microscopy in peripheral smear.

Materials And Methods: Aloe vera leaves were collected, filleted, grinded and filtered using micro filter .Blood samples were taken from 10 people and peripheral smear was done on the glass slide. The extracted gel is then used as immersion medium in peripheral smears. Evaluation was done. Morphological alterations, nuclear details, cytoplasm details, background, artifacts, debris, airbubbles, ease of cleaning were compared with Cedar wood oil and Aloe Vera gel in microscope for peripheral smear.

Results: The results were tabulated. Student t test was used for comparing the various parameters like morphological alterations, nuclear details, cytoplasmic details, background, air bubbles, artifacts .debris and ease of cleaning. Even though the results were no statistically significant aloe vera showed better cytoplasmic details than cedar wood oil and the average value for ease of cleaning for aloe vera and cedar wood oil is 2, 1 respectively.

Keywords: Immersion oil, peripheral blood smear, aloe vera gel, refractive index

INTRODUCTION

Oil immersion lens is one of the microscope lenses with wide uses in medical laboratory filed. It used to magnify the smallest things and for total differential count of blood and for some important infectious diseases also such as bacteria, parasite, but use of lens without oil make the image imperfect and unclear, so to make a better image scientists used immersion oils lens to avoid bending of light **[1, 2]**. A clear image is possible if the available light is directed through the microscope optics to the viewer's eye. Immersion oils play an essential role in maximizing the amount of light producing the image the viewer observes.

Immersion oil with refractive index of 1.515 matches that of the crown glass of oil immersion objectives. Thickened cedar wood oil is prone to drying and could be difficult to remove if allowed to do so. Immersion oils manufactured in the United States contained substantial amount of polychlorinated biphenyls (PCBs), which is considered to have highly dangerous toxins (Nashel and Fischmann 1983) **[3]**.

Aloe vera, a member of the Liliaceous family with a cactus-like appearance isa popular houseplant with a long history as a multipurpose folk remedy. Aloe vera gel mainly has antifungal anti-inflammatory **[4]** hepatoprotective potential due to which it is in major demand in the herbal area of research **[5]** Aloe vera gel is a clear yellow/green liquid with refractive index ranges from 1.33- 1.34 and Specific gravity

is 1.00 to1.03. pH Value is ranges from 3.5 to 4.7

Generally cedar wood oil is used and it must be removed from the objective immediately after use otherwise it will penetrate and damage the cement which holds the objective lens. Cedar wood oil can also 'yellow' with age and has a tendency to absorb light in the ultraviolet and blue range of the spectrum. As an alternate to synthetic oils that are costly and damages the oil immersion objective on frequent use **[6]**, Aloe vera gel was tried as it is cheap and easily available. This study focus on the use of Aloevera gel in place of cedar wood oil in oil immersion microscopy.

MATERIALS AND METHODS

Aloe vera gel was extracted by simple procedure, where 2-4 leaves of aloe were cut at about½ inch from the base so as to drain out all the yellow sap material **[7]**. Then leaves were washed with water and filtered. The filtered aloe vera was grinded and filtered using micro filter **(Figure 3)**. The filtrate is collected in eppendorf tube.

To evaluate the quality of aloe vera and cedar wood oil, 10 blood samples were taken and peripheral blood smears were prepared. The peripheral smear was allowed to dry and stained with Leishmans stain **[8-9]** for 4 mins and Leishman buffer was doubled and let for 10 mins. the slide is then washed under running water and allowed to dry.

A drop of aloe vera gel and cedar wood oil were used in the same slide and observed for scoring under 100 x magnifications. Morphological alterations, nuclear details, cytoplasm details, background, artifacts, debris, air bubbles, ease of cleaning were assessed by the observer. Morphological alterations, nuclear details, cytoplasm details were scored as 0, 1, 2 as nil, good, better respectively. The background was scored as 1, 2 as not clear and clear respectively. Artifacts, debris, air bubbles were scored as 0, 1 as absent and present.

RESULT

According to the score given by the observer, student paired t test was done. The P value of cytoplasmic details was 0.0239 (statistically significant) whereas the p value of other parameters **(Table 1, Graph 1)** were above 0.060 (not statistically significant). The average value of ease of cleaning for aloe vera is 2 and cedar wood oil is **(Table 2, Graph 2)**.

Of the 10 samples observed each with aloe vera gel and cedar wood oil as immersion medium, morphological alterations were less in slide with cedar wood oil and aloe vera gel; Nuclear details, cytoplasmic details, and background of the image were more evident and clear in slide with cedar wood oil when compared to aloe veragel.

Aloe vera gel showed better ease of cleaning compared to cedar wood oil. It was observed that air bubbles and debris were present in slide with aloe vera gel than in slide with cedar wood oil and cedar wood oil had lesser artefacts than aloe vera gel .thus aloe vera can also be used as immersion oil but with little modifications.

Figure 1: Aloe Vera Plant

Figure 2: Aloe Vera Gel Filtered Using Microfilter

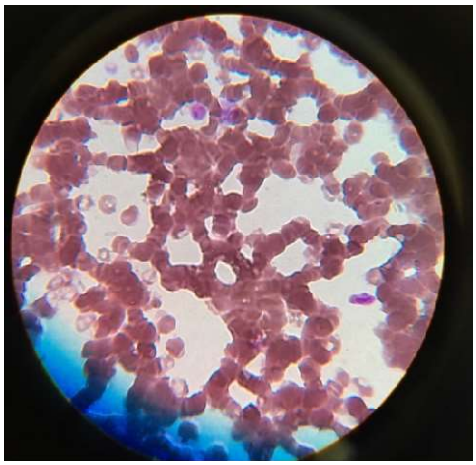
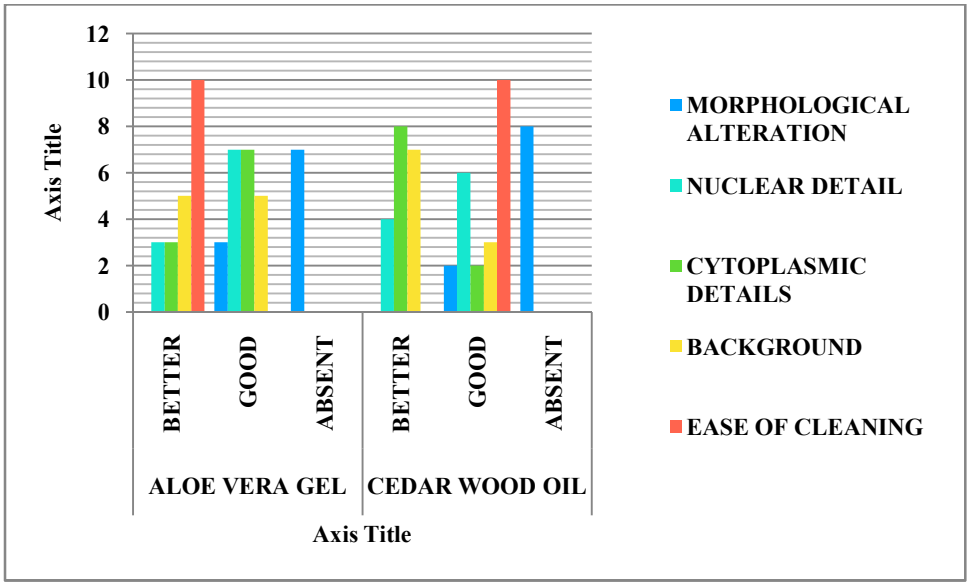


Figure 3: Peripheral Blood Smear Viewed Under 100x Ising Cedar Wood Oil As Immersion Oil

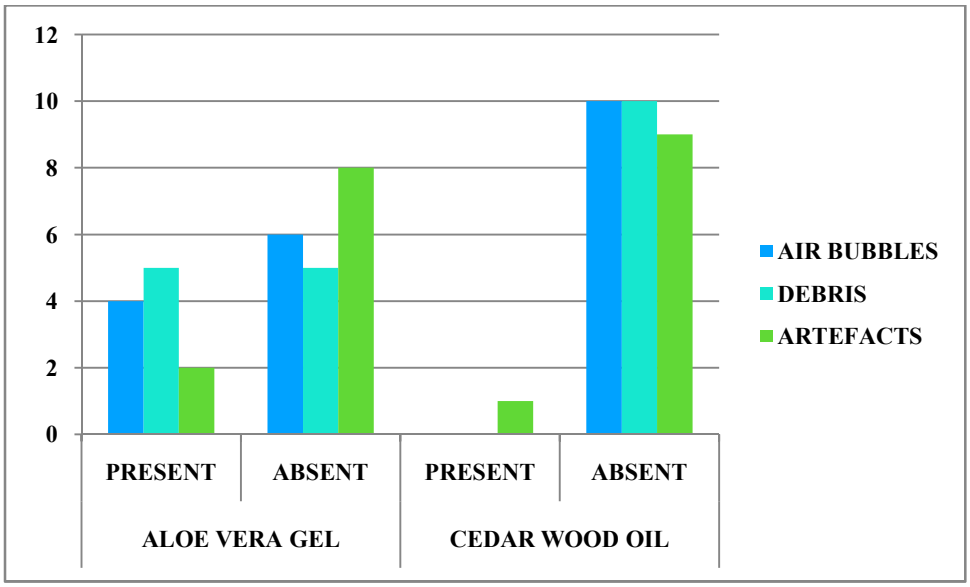
Figure 4: Peripheral Bolled Smear Viewed Under 100x Using Aloe Vera Gel As Immersion Oil



Graph 1

Table 1

	ALOE VERA GEL			CEDAR WOOD OIL		
	BETTER	GOOD	ABSENT	BETTER	GOOD	ABSENT
MORPHOLOGICAL ALTERATION	0	3	7	0	2	8
NUCLEAR DETAIL	3	7	0	4	6	0
CYTOPLASMIC DETAILS	3	7	0	8	2	0
BACKGROUND	5	5	0	7	3	0
EASE OF CLEANING	10	0	0	0	10	0



Graph 2

Table 2

	ALOE VERA GEL		CEDAR WOOD OIL	
	PRESENT	ABSENT	PRESENT	ABSENT
AIR BUBBLES	4	6	0	10
DEBRIS	5	5	0	10
ARTEFACTS	2	8	1	9

DISCUSSION

The immersion oil used must have proper refractive index and density while using oil immersion medium in viewing the peripheral smear. The velocity of light passing through the objective is decreased as compared to its velocity in the air. The ratio of velocity of light in air to that in lens is known as refractive index. Refraction depends on the angle at which the ray strikes the surface of the lens and the refractive index of the medium. Light bends when immersion oil is used. The commercially available cedar wood oil has a refractive index 1.49-1.51. The physical properties of aloevera gel has a refractive index of 1.33, specific gravity of 1.00 and pH of 3.5 to 4.7 **[6]**.

Peripheral blood smear from 10 patients were taken and attained using Leishmans stains. The stained slides were observed under 100x magnification using aloe veragel, and cedar wood oil as immersion oil in the same slide. The slides were scores as 0, 1, 2 based on the morphological alterations, nuclear details, cytoplasmic details, background of the image as nil good and better. The other parameters like presence of air bubbles, artifacts', debris were scored as 0, 1 as absent and present.

While comparing aloe vera gel and cedar wood oil as immersion medium it was noted that morphological alteration were similar for both .The nuclear details and cytoplasmic details were compared based on the morphological shape and stain the nucleus and cytoplasm takes up. It was observed that the nucleus and cytoplasm were more evident and clear in slide with cedar wood oil. The background of the image was clearer in the slide with cedar wood oil when compared with aloe veragel. It was observed that air bubbles and debris were present in slide with aloe vera gel than in slide with cedar wood oil and cedar wood oil had lesser artifacts' than aloe veragel. Aloevera gel showed better ease of cleaning compared to cedar wood oil

CONCLUSION

Although cedar wood oil showed better image characteristics when compared to that of aloe vera gel it can also be used as it's is easier to clean and is cost effective.

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