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# Assessment of the ability of extracts from Lawsonia inermis (Henna) as stains for cytology smears

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***Abstract: Background****: Lawsonia inermis (henna) is a naturally occurring plant. The leaves of the henna plant are the source of red-brown dye widely known as a cosmetic agent. Mahalbiah and Serratia are famous henna oil. They- are consisting perfume, propylene glycol, geraniol and coumarin. Objective: The present study was to assess the henna ability in staining the cytoplasm and nucleus, color of henna in different extraction solvents, effect of henna on morphology of the cell, the effect of henna on nucleus with Mayer's and Haris's Hematoxylin and the effect of mahalbiah and serratia with henna in different extraction. Materials and Methods: The study was conducted at Wad Medani College of Medical Sciences and Technology, Medical Laboratory Science, Department of Histopathology and Cytology. This study was carried during December 2019 to December 2020. 41 Buccal smears stained by using hematoxylin and eosin method for cytological staining and replacing the eosin by the henna solutions (for 20 minutes) and added Mahalbiah and Serratia in method as step in stain for 10 minutes one time before solution (third one without mordant) and one time after solution. The data was analyzed using Scoring analysis by blinded person Does not have a background about the natural dyes which we used in the research.Results: The extraction of henna with absolute ethanol for cytoplasmic stain had been prevented by Harris's hematoxylin and gave blue cytoplasmic color, but in case of Mayer's hematoxylin gave dark brown cytoplasmic color. The extraction of henna didn't affect in nucleus stain with different type of hematoxylin (Mayer's and Harris's) and gave different score with different additives and different ways to apply the additives, the highest score recorded when Serratia applied before henna in stain. The extraction of henna with different additives didn't affect in nucleus stain with Mayer's hematoxylin.. The extraction of henna with normal saline for cytoplasmatic stain gave different score with different additives and different ways to apply the additives , the same extracted solution gave different result for nucleus and cytoplasm which improved with time. Henna extraction with normal saline, different additive and different way of apply them affected on score of nucleus with Mayer's hematoxylin. Henna with tap water only, and Mahalbiah with different ways of apply it gave the same score for cytoplasmatic stain, but in the case of using Serratia as additive with different ways of Applying it gave the same score for cytoplasm. Nucleus score with Mayer's hematoxylin gave different score with different additive. Conclusion: Henna with different additives (Mahalbiah and Serratia ) and extractions (ethanol absolute, normal saline, tap water) gave good score for cytoplasmic stain , but in absolute alcoholic extraction with serratia (applied before henna) gave the best score for cytoplasm and nucleus (stained with Mayer's hematoxylin) and didn't affect in cell morphology.*

**Keywords:** Lawsonia inermis,,Mahalbiah , Serratia.

## Introduction:

Histology is the study of the tissues of the body and how these tissues are arranged to constitute organs (AnthonyL Mescher, 2013). Exfoliative Cytology is the study of cells that have been shed or removed from the epithelial surface of various organs (Dr. Cherian Varghes *et al.*, 2005). A buccal smear is the painless removal of a sample of cells from the inside of your mouth (cheek) for study. (Chernecky CC *et al.*, 2013) Hematoxylin behaves like a basic dye, staining basophilic tissue components, stains the cell nuclei blue-black. Eosin acid dye stains the acidophilic components (cytoplasm) in varying shades and intensities of pink, orange, and red (AnthonyL. Mescher, 2013). Lawsonia inermis (henna) is a naturally occurring plant. The leaves of the henna plant are the source of red-brown dye widely known as a cosmetic agent (James Olayiwola *et al.*, 2017). Mahalbiah and Serratia are famous henna oil. They- are consisting perfume, propylene glycol, geraniol and coumarin (The Islamic place, 2019).

## Materials and Methods:

**Study Design and Duration:** Experimental study analytical was conducted from December 2019 to December 2020.

**Study Setting:** This study was conducted in Wad Medani College of Medical Sciences and Technology, Medical Laboratory Science, Department of Histopathology and Cytology

**Study Population, Sampling technique and sample size:** This study was conducted smear on each of researchers in MST - 41 Buccal smears according to extracted solution.

Pilot study: We bought the henna powder, Mahalbiah and Serratia from the local market. We used sensitive balance and cylinder to measure the amount of henna and solvent which used in this case absolute Ethanol, Normal saline, tap water as solvents to extract the dye, separately, we dissolved the 360g of henna in different solutions (120g in 1500ml tap water, 120g in 500ml normal saline, and 120g in 750 ml absolute Ethanol), we divided the each mixture into three equal parts, adding to one of them 10 ml Mahalbiah and 10 ml Serratia to the other one and third one without mordant. We incubated the mixture for 10 days, and then filtered the mixture by filter paper to be ready for staining. We used hematoxylin and eosin method for cytological staining and replacing the eosin by the solutions (for 20 minutes) and added Mahalbiah and Serratia in method as step in stain for 10 minutes one time before solution (third one without mordant) and one time after solution, and gave good cytoplasmic stain. Best result was obtained in alcohol extraction which applied Serratia before henna. (Hikmat mat Ulah Jah *et al*.,2011) was extracted dye of henna with 1%, 5% and 10% solution using solvents water, ethanol and clove oil.

**Data analyses:** Scoring analysis by blinded person Does not have a background about the natural dyes which we used in the research.

**Results:**
Natural stains as alternative to routine Hematoxylin and Eosin stain in demonstration cytoplasm and nucleus in cytology specimen (41buccal smear).

**Table (1) Eosin with two type of Haematoxylin stain:**

| Stain | Nucleus Score | Cytoplasmic Score |
|---|---|---|
| Harris's Hematoxylin | 9 | 8 |
| Mayer's Hematoxylin | 9 | 7 |

The score of Harris's and Mayer's hematoxylin for nucleus with eosin for cytoplasm gave the same score for the nucleus, and eosin gave different score with different type of hematoxylin. **Tables (2) Lawsonia inermis (henna) stain:**

**Table (2.1) Absolute ethanol used as extraction:**

| **Stain** | **Cytoplasmic Score** | **Nucleus Score** |
|---|---|---|
| **A:** Henna (absolute ethanol) with Harriss hematoxylin. | 7 | 9 |
| **B:** Henna (absolute ethanol) with Mayers hematoxylin. | 8 | 9 |

The extraction of henna with absolute ethanol for cytoplasmic stain had been prevented by Harris's hematoxylin and gave blue cytoplasmic color, but in case of Mayer's hematoxylin gave dark brown cytoplasmic color. The extraction of henna didn't affect in nucleus stain with different type of hematoxylin (Mayer's and Harris's).

**Table (2.2) Absolute ethanol used as extraction with additives:**

| Stain | Cytoplasmic Score | Nucleus Score with Mayer's Hematoxylin |
|---|---|---|
| **C:** Henna (absolute ethanol +Mahalbiah). | 8 | 9 |
| **D:** Henna (absolute ethanol) Mahalbiah applied after henna. | 7 | 9 |
| **E:** Henna (absolute ethanol) Mahalbiah applied before henna. | 8 | 9 |
| **F:** Henna (absolute ethanol +Serratia). | 7 | 9 |
| **G:** Henna (absolute ethanol) Serratia applied after henna. | 7 | 9 |
| **H:** Henna (absolute ethanol) Serratia applied before henna. | 9 | 9 |

The extraction of henna with absolute ethanol for cytoplasmatic stain gave different score with different additives and different ways to apply the additives, in case of stains (D, F ,G) different additive gave the same score, stains (C ,E ) same additive with different ways of apply it gave the same score, the highest score recorded when Serratia applied before henna in stain (H). The extraction of henna with different additives didn't affect in nucleus stain with Mayer's hematoxylin.

**Table (2.3) Normal saline use as extraction:**

| Stain | Cytoplasmic Score | Nucleus score with Mayer's Hematoxylin |
|---|---|---|
| **A:** Henna (normal saline). | 8 | 8 |
| **B:** Henna (normal saline +Mahalbiah). | 7 | 6 |
| **C:** Henna (normal saline) Mahalbiah applied after henna (after1 day from filtration). | 7 | 7 |
| **D:** Henna (normal saline) Mahalbiah applied after henna (after5 days from filtration). | 8 | 8 |
| **E:** Henna (normal saline) Mahalbiah applied before henna. | 7 | 9 |
| **F:** Henna (normal saline +Serratia). | 7 | 8 |
| **G:** Henna (normal saline) Serratia applied after henna. | 8 | 7 |
| **H:** Henna (normal saline) Serratia applied before henna. | 7 | 9 |

The extraction of henna with normal saline for cytoplasmatic stain gave different score with different additives and different ways to apply the additives, in case of stains (B, C, E, F, H) different additive gave the same score, stains (A, D, G) with and without additive gave the same score. According to stains (C, D) the same extracted solution gave different result for nucleus and cytoplasm which improved with time. Henna extraction with normal saline, different additive and different way of apply them affected on score of nucleus with Mayer's hematoxylin, but in case of stain(H)the score of nucleus with Mayer's hematoxylin didn't affect.

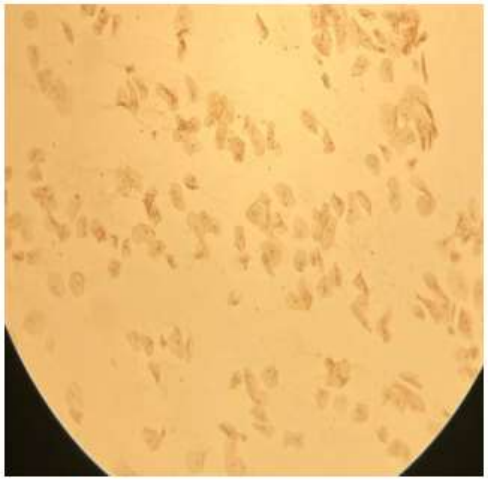
**Table (2.4) Tap water used as extraction:**

| Stain | Cytoplasmic Score | Nucleus score with Mayer's Hematoxylin |
|---|---|---|
| **A:** Henna (tap water). | 7 | 8 |
| **B:** Henna (tap water +Mahalbiah). | 7 | 7 |
| **C:** Henna (tap water) Mahalbiah applied after henna. | 7 | 7 |
| **D:** Henna (tap water) Mahalbiah applied before henna. | 7 | 6 |
| **E:** Henna (tap water +Serratia). | 8 | 7 |
| **F:** Henna (tap water) Serratia applied after henna. | 8 | 8 |
| **G:** Henna (tap water) Serratia applied before henna. | 8 | 8 |

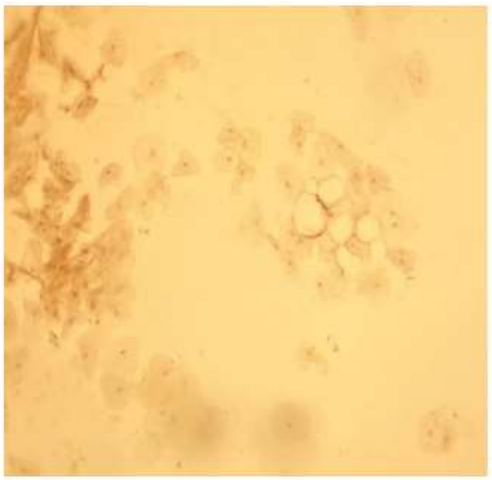
According to stains (A, B, C, D) with tap water only, and Mahalbiah with different ways of apply it gave the same score for cytoplasmatic stain, but in the case of using Serratia as additive with different ways of Applying it gave the same score for cytoplasm. Nucleus score with Mayer's hematoxylin gave different score with different additive.

**Microphotography (1): Lawsonia inermis (henna), ethanol used as extracted**:

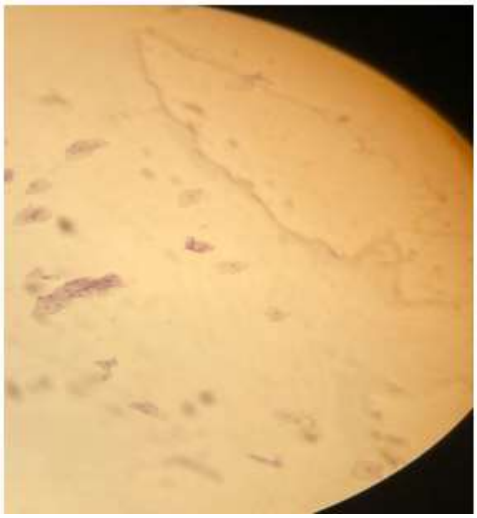
**A:** Henna (absolute ethanol) with Harris's hematoxylin



**B:** Henna absolute ethanol with Mayer's hematoxylin.

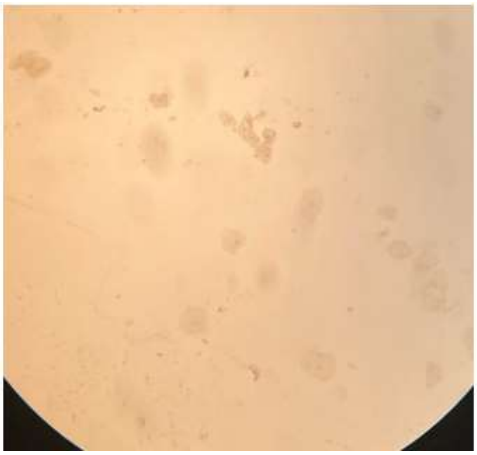


**C:** Henna (absolute ethanol+ mahalbiah) with mayres hematoxylin.

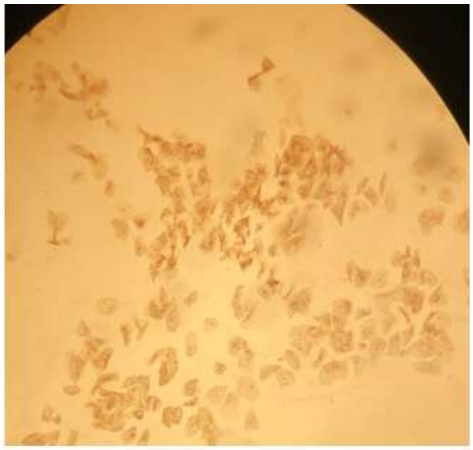


**D:** Henna (absolute ethanol) Mahalbiah applied after henna with mayres hematoxylin

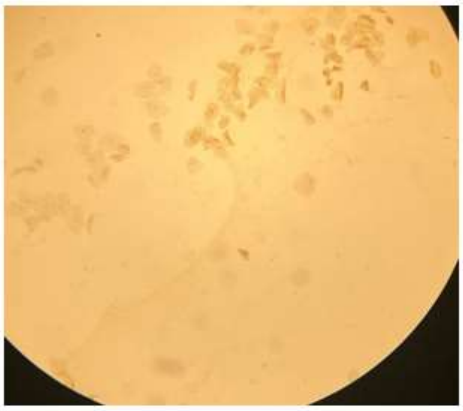
**E:** Henna (absolute ethanol) mahalbiah applied before henna with mayres Hematoxylin.



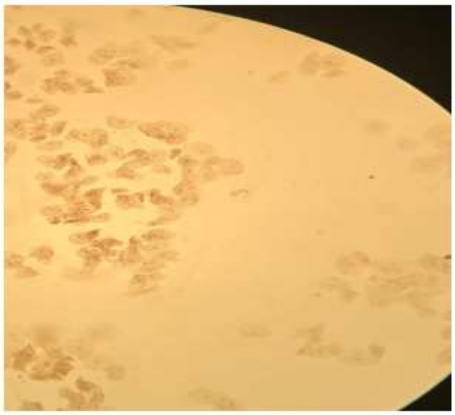
**F:** Henna (absolute ethanol+serratia) with mayres hematoxylin.



**G:** Henna( absolute ethanol) serratia applied after henna with mayers hematoxylin.
With mayers hematoxylin.

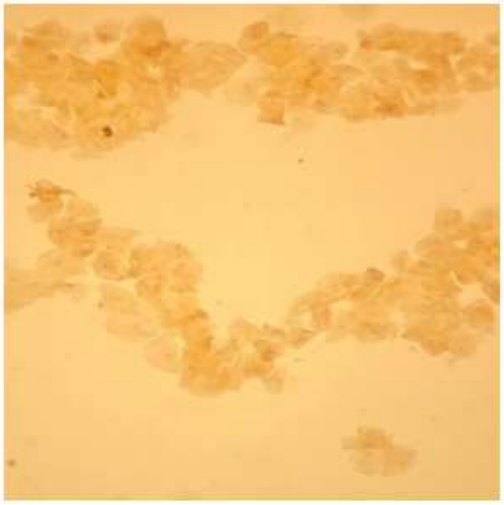


**H:**Henna (absolute ethanol) Serratia applied before henna

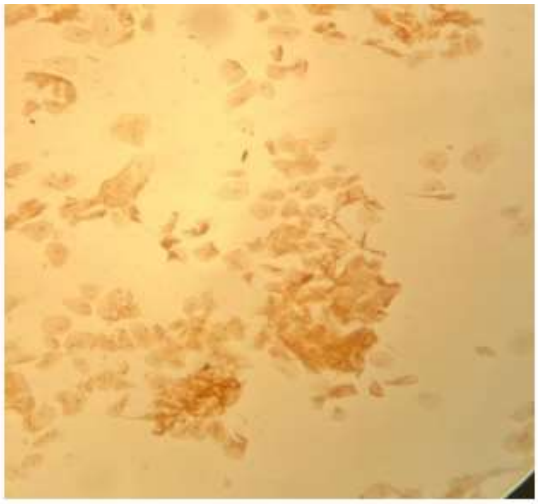


**Microphotography (2): Lawsonia inermis ( henna) , normal saline use as extraction:**

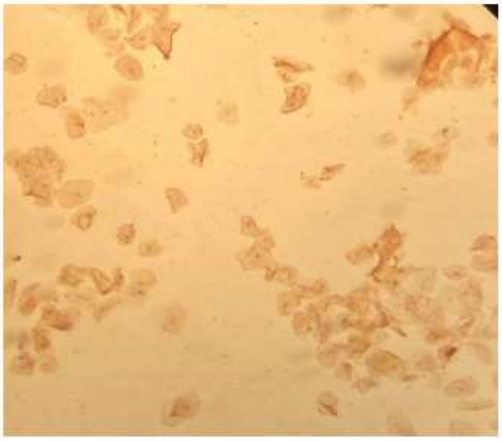
**A:**Henna (normal saline )



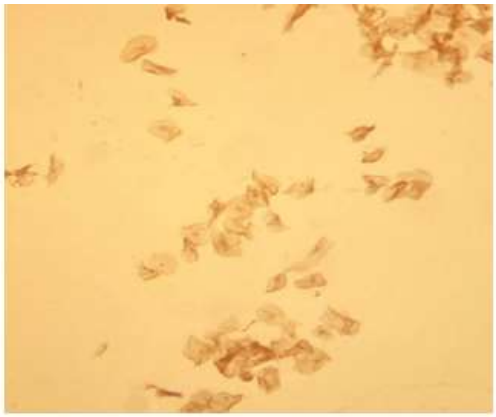
**B:**Henna (normal Saline+mahalbiah)



**C:**enna (normal saline) mahalbiah Applied after henna (after 1 day From filtration)

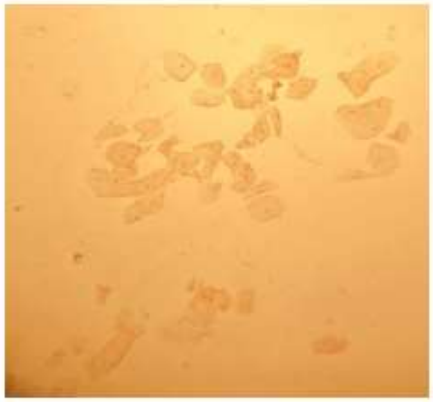


**D:**Henna (normal saline) mahalbiah applied after henna (after 5 days from filtration)

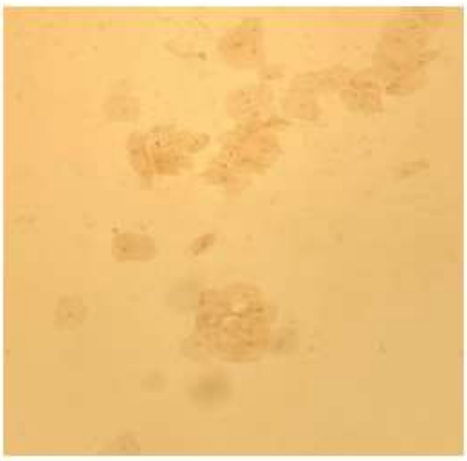


**E:** henna (normal saline) mahalbiah applied before henna.

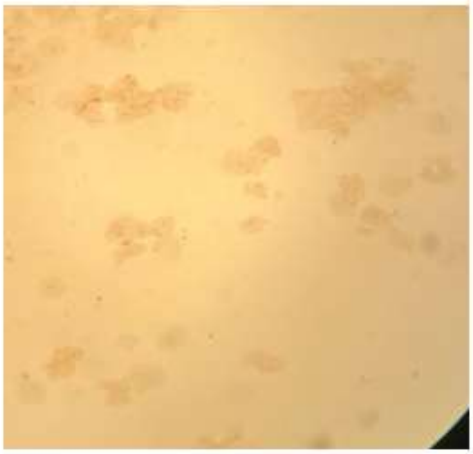
**F:** Henna (norml saline+ serratia)



**G:** Henna (normal saline) serratia applied after henna



**H:** Henna (normal saline) serratia applied before henna



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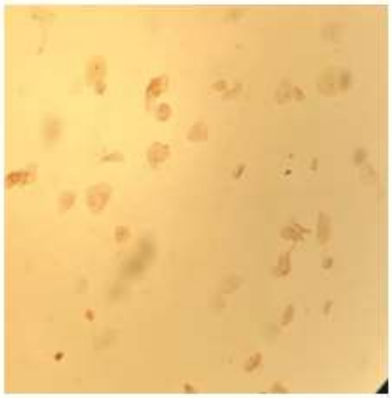
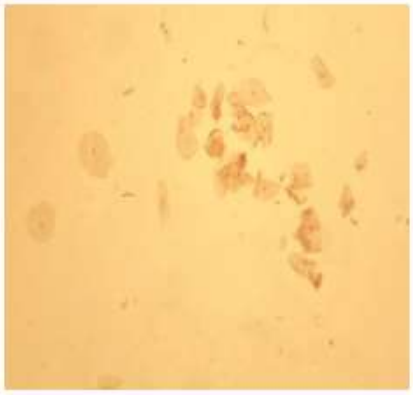
**Microphotography (3): Lawsonia inermis ( henna) , tap water used as extraction :**

**A:** Henna (tap water)



**B:** Henna( tap water+ mahalbiah)

**C:**Henna (tap water) mahlbiah applied after henna.



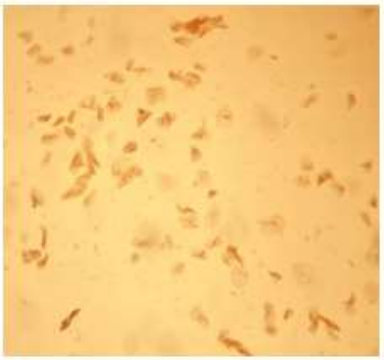
**D:**Henna (tap water) Mahalbiah applied Before henna.

**E:** Henna (tap water+serratia)

serratia

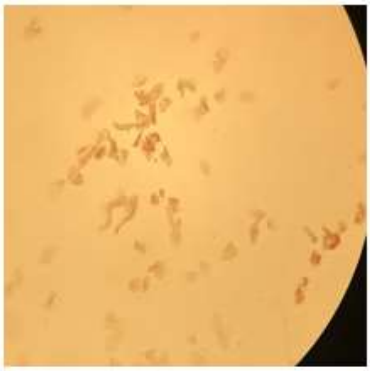
**F:** Henna (tap water)

Applied after henna



**G:** Henna (tap water) serratia applied

Before henna.



**Discussion:**

Staining has become a vital part in histology. In this study, we are sorted to using local natural alternatives dye that contribute to support the economy and reducing the financial burdens on citizens in obtaining health services and serving medical workers by reducing risks related to synthetic dye. Hence, we thought of exploring naturally available stains that could substitute eosin. Lawsonia inermis (Henna) has been widely used as an ornamental stain for hair, skin, and nails giving a reddish orange to brown color. In the preparation of henna the working staining solutions we used ethanolic and tap water extractions , but ethanolic extract dissolved completely with higher volume than tap water which incompletely dissolved and gave lower volume, but in James Olayiwola study the aqueous extract dissolves completely but the ethanolic extract always leave a residue implying that within a given volume, saturation is attained earlier (James Olayiwola Adisa et al., 2017) ,May be attributed to using powder instead of using leave. In our study we used ethanolic and tap water extractions but the alcoholic extraction of henna gave dark solution while tap water extraction gave green solution, But in Hikmat study the color of the dye extracted from Lawsonia leaves with water was dark brown while that extracted with ethanol and clove oil were light brown (HIKMAT ULLAH JAN et al., 2011), but in our study, we also disagreement with (HIKMAT ULLAH JAN et al., 2011) by using normal saline extraction and gave brown solution. May be attributed to use henna as powder for long incubation period to extract the color. We also observed that as long as we remained the extracted solution getting more darker in color, and filtration of henna powder is more difficult in tap water and normal saline extraction than alcoholic extraction. In our study the three different extractions ( absolute ethanol, tap water , normal saline ) which used to extract the color gave different cytoplasmic color from (dark brown ,light brown , brown) spontaneously, In James study Three different concentrations (0.5%, 1% and 2%) were used and both the aqueous and ethanolic extract stains the cytoplasm golden brown, 1% concentration gave the best effect on liver biopsies (hepatocytes) (James Olayiwola Adisa et al., 2017) we disagreement with (James Olayiwola Adisa et al., 2017), in using normal saline extraction. and gave good score, and the best effect on cytological sample (buccal)by alcoholic (100% ethanol) extraction got score (9), may be attributed to use alcoholic extraction with higher concentration

100% in cytological sample for long period of incubation. In our study, we added Mahalbiah and Serratia to henna extractions, in case of normal saline extraction the additive solution didn't enhance henna stainability, but in case of alcoholic and tap water extraction Serratia enhance henna stainability. But in James study the result of treatment with potassium alum (mordant) has no observable effect on the staining reaction with Henna extract. It was observed that simple Henna solutions without mordanting stained the tissues better and the addition of a mordant does not improve its staining qualities. Three different concentrations of phenol (an accentuator) (1%, 3% and 5%) were added to Henna staining solutions to see if it can improve the staining reaction of the stain. 1% and 3% appears to have a positive effect on staining with Henna especially with the ethanolic extract, Phenol used as an accentuator has been found to improve the staining but caused swelling of cell. Mordanting with potassium alum did not cause swelling but did not give the kind of resolution as obtained with Phenol. (James Olayiwola Adisa et al., 2017), and in Judith study henna was used as a pure extract dissolved in lemon juice to stain brain tissue. This produced a temporary stain that stained tissue brown, but faded with time. However, when mordanted with alum (but not tin or iron), dye affinity to the tissue was enhanced without changing its reaction as an acidic stain. This resulted in a permanent stain that did not fade with time. (Judith N. Alawa et al., 2015). The three extracted solutions of henna gave clear, stable cytoplasmic color and didn't fade with time, when staining in different days the filtered solutions get darker as solution and the color of the cytoplasm became deeper than the day before as mentioned in stains (C: Henna (normal saline) Mahalbiah applied after henna after1 day from filtration and D: Henna (normal saline) Mahalbiah applied after henna after5 days from filtration). Instead of using (Phenol, potassium alum, lemon juice) in histological stains we used Mahalbiah and Serratia as additive to henna solutions in cytological stains. Comparing the result of henna according to tables (4.1.3.1) (4.1.3.2) (4.1.3.3) (4.1.3.4) in alcoholic extraction Mahalbiah and Serratia enhanced cytoplasm stainability specially in stain (H) and get score (9). while this additive didn't affect in nucleus score with Mayer's hematoxylin score (9). In case of normal saline extraction, the additive didn't improve cytoplasm stainability but effect in score of nucleus with Mayer's hematoxylin. In case of tap water extraction Serratia (E, F, G) increase stainability of cytoplasm and effect in sore of nucleus with Mayer's hematoxylin. We observed that the higher score for different extractions and different additive recorded by alcoholic extraction with Serratia in slide (H) get score (9). In alcoholic extraction the score of nucleus with Mayer's hematoxylin gave the same result with different additive ( Mahalbiah and Serratia) (9), in normal saline and tap water extraction effected on Mayer's hematoxylin staining of the nucleus to give different score (6,7,8,9) of nucleus with Mayer's hematoxylin stain according to different additive specially in case of using mahlabiah as additive that give the lowest score. The Harris's hematoxylin effected the cytoplasmic stain with henna extractions and in case of alcoholic extraction which was preventing the cytoplasmic stain with henna stain (A: Henna absolute ethanol with Harriss hematoxylin). Mayer's hematoxylin with alcoholic extraction of henna had a similar score to Mayer's hematoxylin with eosin (9), but in stains (B, C, E, H) henna extraction got higher score than eosin (7) and (H) slide got the highest score (9). In normal saline extraction stains (E, H) had a similar score of Mayer's Hematoxylin with eosin (9), but stains (A, D, G) had higher score than Eosin (7). While in case of tap water extraction the Myer's hematoxylin got the lower score (6) comparing to Myer's hematoxylin and eosin, but in stains (E, F, G) had higher score than eosin (7). In alcoholic extraction stain (H) got higher score (9) than eosin with Harris's Hematoxylin (8).

**Conclusion:**

Henna with different additives ( Mahalbiah and Serratia ) and extractions (ethanol absolute, normal saline, tap water) gave good score for cytoplasmic stain , but in absolute alcoholic extraction with serratia( applied before henna) gave the best score for cytoplasm and nucleus (stained with Mayer's hematoxylin) and didn't affect in cell morphology.

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