

# A SYSTEM FOR RAPID LOCALIZATION AND INTENSITY EVALUATION OF SAPONINS ACCUMULATION IN PLANT TISSUES

<sup>1</sup>MELA G. ABDUL-HALEEM, <sup>2</sup>Dr. LOAY E. GEORGE, <sup>3</sup>Dr. ANSAM G. ABDUL-HALEEM

<sup>1,2</sup>Department of Computer Science, University of Baghdad, Iraq

<sup>3</sup>Department of Biology Science, University of Baghdad, Iraq

E-mail: <sup>1</sup>a.mayla@yahoo.com, <sup>2</sup>Loayedwar57@scbaghdad.edu.iq, <sup>3</sup>ansamghazi@yahoo.com

## ABSTRACT

A fluorescence microscopy considered as a powerful imaging tool in biology and medicine. In addition to useful signal obtained from fluorescence microscopy, there are some defects in its images such as random variation in brightness, noise that caused by photon detection and some background pixels in the acquired fluorescence microscopic images appear wrongly auto-fluorescence property. All these practical limitations have a negative impact on the correct vision and analysis of the fluorescent microscope users. Our research enters the field of automation of image processing and image analysis using image processing techniques and applying this processing and analysis on one of the very important experiments in biology science. This research is devoted to develop a system based on digital image processing methodology to localize and assess the concentration of saponins accumulation in plant tissues using Fluorescence microscopic image. The proposed system involved preprocessing steps than to make the region of interest more obvious and reflects the saponins accumulation area. Also, the system introduces a simple mathematical way for concentration assessment, and it was justified through the test results. It includes building a system to get microscopic images with best appearance and no defects. It determines the saponins accumulation sites in leaves, rhizomes and shoot apex of *Y. gloriosa* Variegata and their *in vitro* cultured tissues (Calli, direct and indirect regenerated shoots and rhizomes/roots). Statistical analysis is performed using a computer to get the mean and median of saponins intensities in each part, and finally perform a comparison between them to determine which part can record the highest intensity level of saponins. The results showed high ability of the system to determine the locations and intensity of saponins in the different parts of the plant. It performs the statistical analysis very quickly. In *in vitro* culture, it was found that callus treated with Thidiazuron (TDZ) in a combination with benzyl aminopurine (BA) and naphthyl acetic acid (NAA) after 3 weeks of culture had the highest level of saponins accumulation, while the leaves of intact plant recorded the second highest accumulation of saponins.

**Keywords:** *Fluorescence Microscopy, Histogram Statistics, Contrast Stretching, region of interest (ROI), Saponins intensity*

## 1. INTRODUCTION

Microscopy imaging includes fluorescence microscopy and electron microscopy, has taken a prominent role in life science research and medicine due to its ability to investigate the 3D interior of live cells and organisms [1]. One essential advantage of fluorescence microscopy is the presence of fluorescent molecules themselves (Auto-fluorescence). Auto-fluorescence is a phenomenon that consists of the emission of light in the visible spectral range of ultraviolet radiation,

when biological substrates affected by light at an appropriate wavelength. On the other hand, specific fluorochromes can be added to the specimen, depending on what is being investigated [2].

To obtain a fluorescent image from fluorescence microscopy, there are four basic processes which include fluorophore excitation, Photon emission, Photon detection and quantification and storage. After the specimen has been prepared and is putting underneath the microscope, the fluorescent molecules

(fluorophores) must be converted into an excited state by absorption of a photon. This is done by illuminating the specimen, with a lamp or laser. Then each fluorophore may emit a photon with a lower energy (i.e. longer wavelength) than the photon previously absorbed. A part of the light should enter the objective lens of the microscope and be focused towards a detector. When a photon hits the detector, it releases an electron. After a fixed time interval, the charges of a set of such electrons are quantified, and pixel values are determined from these quantified. A larger charge is translated into a higher pixel value [3].

Although, the big advantages of using fluorescent microscope in biological domain, but like all techniques, it is subject to practical limitations. The recent developments with fluorescent microscopy concern with improvement of image quality by addressing the basic problem of image resolution. It is determined by image contrast (i.e., Uncertainty in brightness), the diffraction of light within optical systems that results in blurring the image and the noise that can be formed from imprecisions when the charge of small clouds of electrons are determined quickly. In fact, the emission of the photons is itself random, so we would still get noisy images even if we detected every photon perfectly [3, 4]. To overcome on all of these limitations the image processing techniques are used. As well as, Image processing techniques provides more advantages than traditional methods of analysis: 1) In spite of the human vision is very sensitive, but it can be easily biased from preconceived notions of objects and concepts; while automated image analysis provides an unbiased approach to extract information from image data and test assumptions. 2) Once a routine has been developed for image analysis, it can be applied to a large number of microscopic images, so, image processing techniques facilitate the collection of large amounts of data for statistical analysis.

In this research, many image processing routines are used to overcome on the limitations of fluorescence microscopy. A simple and fast method for saponins regions is introduced. The developed algorithm offers a comprehensive scenario can handle automatically detect the saponins accumulation regions and assess its concentration. Saponins are the most important components of medicinally important plants. The accumulation of these compounds in leaves, rhizomes and shoot apex of *Y. gloriosa* Variegata and their *in vitro* cultured tissues (calli, direct and indirect regenerated shoots and rhizomes/roots) is

performed by calculating the mean and median of intensity values of saponins in these parts.

Steroidal saponins are important active components of *Yucca gloriosa* Variegata, a medicinally important plant. However, most studies on *Y. gloriosa* have mainly focused on the components analysis, pharmacology and medicinal treatments. Saponins are glycosylated natural products, a vast group of structurally diverse glycosides of the plant kingdom and also of some marine organisms and insects [5]. They have a high molecular weight. They are able to interact with cell membranes and are able to decrease the surface tension of an aqueous solution [6], and these surface active properties distinguished them from other glycosides, beneficiary from them in industrial applications as foaming and surface active agents. They contain a steroidal or triterpenoid aglycone and one or more sugar chains. The detection and localization of saponins accumulation are very necessary to determine which plant organ or tissue accumulates high levels of such important compounds.

Some of related works are shown in the next section. A brief description for basic tasks involved in the proposed system is introduced in section 3. The results of the image enhancement and isolation of saponins sites, and the mean and median values for saponins intensity are explained in the experimental data and results section. Finally, the main conclusions derived in this work and a list of recommendations for future works are presented in the conclusion section.

## 2. RELATED WORK

Some of the published works related to image processing and fluorescence microscopy techniques are given in the following paragraphs:

Du and Dua [7], used k-means clustering, EM, Otsu's threshold, and GMAC methods for unsupervised learning in cell image segmentation. A comparison were performed between these methods are compared to show strengths of efficiency and embedded limitations.

Matula [8] concerned with digital image processing in fluorescence microscopy and its use in cell biological experiments. The work was conceived as a collection of 26 scientific articles divided into four groups according to areas that they contribute to (1) automation of image acquisition and image analysis, (2) image segmentation, (3) object tracking and (4) applications in the cell nucleus research.

Kervrann *et al.* [1] introduced advances in fluorescence and electron microscopy and determined the image processing and analysis methods required to quantify phenotypes for a limited number but typical studies in cell imaging.

Chang *et al.* [9], analyzed proliferation, kinematics and attachment of mouse embryonic stem cells using time-series confocal time-lapse fluorescence microscopy images by using an automated image processing technique. The results of the implementation showed that the automatic method can successfully analyze cell kinetics, proliferation and attachment.

### 3. SYSTEM LAYOUT

The proposed system has the essential stages to perform all relevant pre-processing, segmentation (i.e., ROI isolation) and statistical analysis tasks. The general structure of the proposed model is shown in Figure (1).

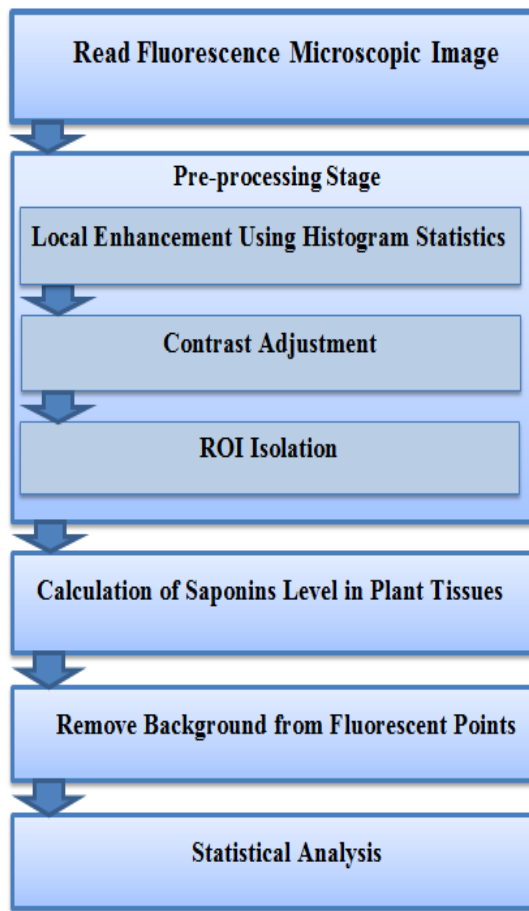


Figure 1: The proposed model architecture.

As clarified in Figure 1, the system consists of three stages (i) preprocessing, (ii) allocation the saponins region & (iii) statistical analysis for estimating the level strength of saponins accumulation. All introduced methods a simple and fast, it can be used to accomplish the whole accumulation and assessment task automatically without need for conducting algorithms have high-complicated cost.

#### 2.1 Read Fluorescence Microscopic Images

The fluorescence microscopic image is fed to the system as a Bitmap (BMP) image file; the color resolution of the image is taken 24 bits per pixel (bpp). The image data (i.e. Red, Green, Blue (RGB) components) is loaded.

#### 2.2 Pre-processing Stage

The main focus of this stage is to enhance the image for subsequent pattern recognition and to overcome on the drawbacks of fluorescence microscopy. This phase includes many steps:

##### 2.2.1 Local enhancement using histogram statistics

The histogram statistics are used to enhance details over small areas in an image. For each different intensity value, the histogram represents the number of pixels in an image which have this value. The histogram for 8-bit gray image will graphically display 256 numbers for 256 different possible intensities gray scale values. Histograms can also be taken of color images either by taking the individual histogram of red, green and blue channels, or a three dimensions (3-D) histogram can be produced, with the three axes representing the red, blue and green channels can be taken. The operation includes scanning the image in a single pass and counting the number of pixels found at each intensity value. This is used to construct a suitable histogram [10, 11].

##### 2.2.2 Contrast adjustment

Often, fluorescent microscopic images are dark and have a low dynamic range and many of its features are difficult to see. Detail discrimination relies heavily on contrast, which is the difference in brightness between regions of the same field of view. To improve the contrast in an image, contrast stretching (often called normalization) is used. It works on 'stretching' the range of pixel values to span a desired range of values. To perform the stretching, the upper and lower limits of pixel values limits over which the image is to be normalized must be specified. These limits will be

obtained just by taking the minimum and maximum pixel values that the image type concerned [12]. In this research, the upper and lower pixel value limits are obtained by using the histogram and thresholding technique for blue color.

### A. Gamma Stretching

One of the best intensity transformations that will improve the appearance of the images is gamma contrast adjustment. When preparing imagery for computer display; gamma is the degree of contrast between the mid-level gray values of an image and it does not have any effect on the black or white values of an image. By applying a gamma correction, the overall brightness of an image can be improved. If the gamma value is set too high, middle tones appear too dark; however, if the gamma value is set too low, middle tones appear too light and the image looks bleached out. Additionally, Gamma can change the ratios of red to green to blue [13]. A target color can be determined so the gamma is calculated based on the present average color of the raster and user-specified target color. Algorithm (1) shows the main steps to perform this process. For calculation of best threshold used in calculation of minimum gray level, the value of  $\alpha$  is set to (0.002) while for maximum gray level and it is set to (0.01). Also, the inclusion parameter (E1) in gamma stretching equation for blue color is set to 3.5, and the inclusion parameter (E2) is set to 5.

<b>Algorithm (1) : Histogram Calculation and Gamma Stretching</b>
<b>Goal:</b> intensity transformation
<b>Input:</b> RGB components
<b>Output:</b> new-blue(), new-Green(), new-Red()
<p><b>Step 1:</b> Calculate Histogram for blue color of RGB image.</p> <p>For all pixels of image his(i) ← his(Blue(x,y)) + 1 End For</p> <p><b>Step 2:</b> Calculate the minimum and maximum pixel values that the image type concerned.</p> <p><b>Step 2.1:</b> Calculate minimum value and select a threshold as a small ratio of the image area.</p> <p>Thr ← <math>\alpha * (Wid * Hgt)</math> For all i=0→255 sum ← <math>\sum His(i)</math> check If sum &lt;= Thr Then Min ← i Exit Loop</p>

<p>End if End For</p> <p><b>Step 2.2:</b> Calculate maximum value and select a threshold as a small ratio of area of the image.</p> <p>Thr ← <math>\alpha * (Wid * Hgt)</math> For all i=255→i sum ← <math>\sum His(i)</math> check If sum &gt;= Thr Then Max ← i: Exit Loop : End if End For</p> <p><b>Step 3:</b> Do Gamma filter Stretching for Blue and Green colors.</p> <p>For all i → Min + 1 To Max - 1 Table-B(i) ← <math>\left(\frac{i-Min}{Max-Min}\right)^{E1} * 255</math></p> <p>Table-G(i) ← <math>\left(\frac{i-Min}{Max-Min}\right)^{E2} * 255</math> End For</p> <p><b>Step4:</b> Get the new RGB components.</p> <p>New-Blue(x, y) ← Blue(x, y) + 2 * Table-B (Blue (x, y)) / 3 New-Green(x, y) ← Table-G (Blue (x, y)) New-Red(x, y) ← 0</p> <p><b>Step5:</b> Return New-Blue(), New-Green(), New-Red().</p>
--

### B. Linear Stretching

Histogram of an image is analyzed based upon brightness and contrast of the image. If the histogram of the image is dark, the pixels of the image move towards the lower gray level region. If the histogram of the image is bright, the pixels of the image move towards the higher gray level region. The histogram will not be equally spread if it is a low contrast image. The histogram will be equally spread if it is of high contrast. Linear stretching and histogram equalization are the two methods of histogram manipulation. In linear stretching, the dynamic range of the image is increased while the shape of the histogram is not altered [14]. This step is used to increase the dynamic range of blue color. The process starts with calculation the histogram of blue color of each pixel resulted from the previous step. The minimum and maximum level is obtained in the same way. Then, linear stretching equation (1) is applied. For calculation of best threshold used in calculation of minimum and maximum gray level, the value of  $\alpha$  is set to (0.001) as shown in algorithm (2).

$$g(b) = \left(\frac{f-min}{max-min}\right) * 255 \quad (1)$$

Where  $g$  is blue pixel,  $G(b)$  is the transformation function of linear stretching, the original gray level are [min, max], and the target gray levels are [0, 255].

**Algorithm (2) : linear Stretching**

**Goal:** increase the dynamic range of blue color

**Input:** new-blue(), new-Green(), new-Red()

**Output:** final-blue(), final-Green(), final-Red()

**Step 1:** Calculate Histogram for new blue color of RGB image.

For all pixels of image //x width index, y for height index

his(i) ← his(new-Blue(x,y)) + 1

End For

**Step 2:** Calculate the minimum and maximum pixel values that the image type concerned.

**Step 2.1:** Calculate minimum value and select a threshold as a small ratio of the image area.

Thr ←  $\alpha * (W \text{ id} * \text{Hgt})$

For all i=0→255

sum ←  $\sum His(i)$

check If sum <= Thr Then

Min ← i

Exit Loop

End if

End For

**Step 2.2:** Calculate maximum value and select a threshold as a small ratio of area of the image.

Thr ←  $\alpha * (W \text{ id} * \text{Hgt})$

For all i=255→i

sum ←  $\sum His(i)$

check If sum >= Thr Then

Max ← i: Exit Loop :

End if

End For

**Step 3:** Do linear filter Stretching for Blue and Green colors.

For all i → Min + 1 To Max - 1

Table-B (i) ←  $\left( \frac{f-min}{max-min} \right) * 255$  // equation (1).

End For

**Step4:** Get the new RGB components.

final-Blue(x, y) ← Table-B (new-Blue (x, y))

final-Green(x, y) ← new-Green (x, y)

final-Red(x, y) ← new-Red (x, y)

**Step5:** Return final-Blue (), final-Green(), final-Red(x, y).

This step is used to increase the dynamic range of blue color. The process starts with calculation the histogram of blue color of each pixel resulted from the previous step. The minimum and maximum level is obtained in the same way. Then, linear stretching equation (1) is applied.

**2.3 ROI Isolation**

The ROI extraction is considered as a type of segmentation. After enhancement, the fluorescent region can be seen as a special island in the image. Using the seed filling principle with appropriate constrain lead to optimal segmentation of these regions by removal all other islands in the image. This method includes two steps: the first step is the identification of seeds. In case of island removal the seed point has foreground value. The second step includes constraints to prevent each region from overfilling. In this work, the selected constrain is that the selected filling region in case of segmentation must form very small ratio of the image area (0.3) otherwise it will be ignored. This process is shown in algorithm (3) and algorithm (4).

**Algorithm(3):Seed Filling Function**

**Goal:** The number of pixels in seed region

**Inputs:** p // target pixels

I // x-position of the target pixel

J // y-position of the target pixel

**Output:** SS // the number of pixels in seed filling region

**Step 1 :** Enter the pixel positions in an array Q

**Step 2 :** Initialize pixel count to zero

**Step 3 :** While Q is not empty:

**Step 4 :** Set n equal to the last element of Q.

**Step 5 :** Remove last element from Q.

**Step 6 :** Increase pixel count SS

**Step 7 :** Check If the color of n is equal to target-color:

Check If the west pixel value equal to the target value

Add west pixel to end of Q.

End If

CheckIf the east pixel value equal to the target value

Add east pixel to end of Q.

End If

CheckIf the north pixel value equal to the target value

Add north pixel to end of Q.

End If



```

CheckIf the south pixel value equal to the target
value
    Add south pixel to end of Q.
End If
    End If
End While
Step 8 : Return SS;
    
```

**Algorithm(4): ROI isolation**

**Goal:** fluorescent regions extraction

**Inputs:** final-blue()

**Output:** seg() // island removal image

```

Step 1: Calculate Histogram for blue color of
RGB image.
For all pixels of image //x width index, y
for height index
    his(i) ← his(final-Blue(x,y)) + 1
End For

Step 2: Calculate maximum value and select a
threshold as a small ratio of area of the
image.
Thr ← α * (Wid * Hgt) // α = 0.02
For all i=255→i
    sum ← Σ His(i)
    check If sum >= Thr Then
        Max ← i: Exit Loop :
    End if
End For

Step 3: Select seed point
For all pixels of image
    checkIf final-Blu(x, y) >= Max
Then Tmp(x, y) = 1
End If
End For

Step 4: Enter the constant criteria parameter C

Step 5: Count the region ratio
rat ← C × (Hgt × Wid) // C=0.3

Step 6: Search for each target pixel in all image
and call seed function to each ones
For all pixels in the Tmp image// x as row
number, y as column number
CheckIf pixel value = 1 Then// seed pixel
S ← seed (1, x_position, y_position);
//algorithm (3)

Step 7: Compare the number of pixels in the seed
region with the region ratio
CheckIf S ≤ rat Then// keep region in the
image
For all pixel in the image // i as row
number, j as column number
CheckIf seed region(i,j)=1 Then
    
```

```

        seg(i,j)←255
        else
        seg(i,j)←0
    End If
    End For // i& j
End If
End If
End For // x& y
Step 8: Return seg();
    
```

**2.4 Calculation of Saponins Level in Plant Tissues**

The level of saponins in plant tissues can be determined by calculating the intensity of fluorescence in fluorescent sites which represent the sites of saponins accumulation in these tissues. This process includes obtaining gray image from the original color image. Then, only the gray-level of isolated sites are taken where the value of blue color after preprocessing equal to 255. The other pixel must be ignored (i.e., set to 0). The gray image  $Gray(i,j)$  at position  $(i,j)$  is determined as the average of red, green, and blue bands as shown in equation (2) and algorithm (5).

$$Gray(i,j) = (Red(i,j) + Green(i,j) + Blue(i,j))/3 \quad (2)$$

**Algorithm(5): Saponins level calculation**

**Goal:** saponins intensity

**Inputs:** seg()

**Output:** fluorescent()

```

Step 1: Calculate gray image from the original
RGB image.
For all pixels of image //x width index,
y for height index
    gray(x,y) ←Equation(2)
End For

Step 2: Get the intensity for only fluorescent
points
For all pixels of image //x width index,
y for height index
checkIf seg (x,y) == 0 Then
    fluorescent (x,y) ←0
    Else
    fluorescent (x,y) ← gray(x,y)
End if
End For

Step 3: Return fluorescent ();
    
```

**2.5 Remove Background from Fluorescent Points**

One of the defects of fluorescence microscopy is that the light starting from one point in the specimen cannot be focused back to a one point on the detector. Consequently, it can spread at the end over several pixels [3]. To get actual fluorescent points, the intensity value of each fluorescent pixel is subtracted from a local mean using equation (3). The local mean of each fluorescent pixel is calculated within (17×17) window that surrounding it.

$$nGray(i,j) = Gray(i,j) - Mean(i,j) \quad (3)$$

Where  $nGray(i,j)$  represents the new intensity value of pixel at position  $(i,j)$  and  $Mean(i,j)$  represents its local mean. The applied previously mentioned steps are clarified in Figure (2).

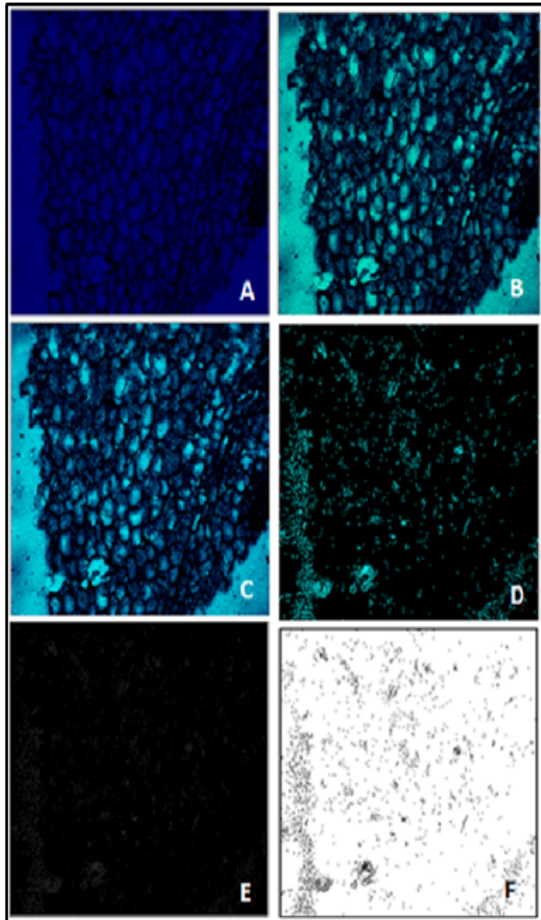


Figure 2: The Pre-processing, ROI Isolation, and Intensity Calculation Processes;  
A: An Image Acquired by a Fluorescence Microscopy; B: Gamma Stretching Effect; C: Linear Stretching Effect; D: ROI Isolation; E: Saponins Intensity Image with Black Background; F: Saponins Intensity Image with White Background for Better View.

## 2.6 Statistical Analysis

The statistical analysis of input image is implemented using two measures like mean and median. The basic of all statistical measurements is mean. It is measured by calculating the average of all pixel values within  $m \times n$  window. While median is the measure of intensity level of pixel. It works by selecting the middle pixel value from the ordered set of values within the ' $m \times n$ ' window around the reference pixel. If the number of values in the set is an even number, then the average of the two values in the middle of the ordered set is taken [15]. Saponins accumulation is identified according to the fluorescent intensity. In this research, the mean and median of the intensity of fluorescent points only is calculated. To calculate mean and median, all fluorescent points are put in one dimension array and then the arithmetic formula of mean is performed as the following:

$$mean = \frac{1}{k} \sum_{i=1}^k wln(i) \quad (4)$$

Where  $k$  represents the number of fluorescent points in the image and  $wln()$  one dimension array of all fluorescent points. To calculate median value, the array  $wln()$  is sorted in ascending order and then the median value is taken as the following:

$$median = Sortedarray(wln(m)), \quad m = k/2 \quad (5)$$

## 3. EXPERIMENT AND RESULTS

A significant variation in the fluorescence and density between the classes of samples that represent anatomical sections of different parts of the intact plant of *Y. gloriosa* Variegata (leaves, rhizomes and shoot apex) and their *in vitro* cultured tissues (Calli, direct and indirect regenerated shoots and rhizomes/roots) were recorded. The fluorescence and density detect the localization of saponins accumulation in these tissues. The number of the taken classes in this research was twelve and each class contained three samples (i.e., thirty-six samples were examined). Each sample passed through all of the system stages until the mean and median values of fluorescent points were recorded as shown in Table (1) and Figure (3). For each class, the averages of means and medians values of the three samples were extracted to represent the level of saponins accumulation in the class. Figure (4) showed that the averages of means and medians values of saponins intensity were very close to each other.

The prior articles that contributed in the field of localization of saponins accumulation in *Y. gloriosa* Variegata plant are very little and they depended on using traditional methods of analysis or on the observed visualization in the fluorescent microscopic images by an expert or a biological researcher. While this research automatically detected on saponins accumulation and an automatic statistical analysis was performed where the decisions were made by a machine. So, only one article will be compared with this contribution.

Abdul-Haleem [16] employed histological analysis and fluorescence microscopy techniques to determine the locations of saponins accumulation in different parts of *Y. gloriosa* Variegata plant. Both thin layer chromatography (TLC) analysis and High performance liquid chromatography (HPLC) analysis of steroidal saponins from *Y. gloriosa* Variegata extracts were used and matched the results with the observed visualization in the images of sections examined by fluorescence microscope. Abdul-Haleem [16] found that callus that treated with TDZ accumulated the highest amount of saponins. The results of this research were compatible with Abdul-Haleem [16] from which the samples were taken. These samples were acquired using UV light microscopy and by employing aniline blue stain to visualize saponins sites.

The highest intensity of saponins was found in callus treated with TDZ in a combination with BA and NAA after 3 weeks where the average of means and medians of saponins intensity were 65.00 and 64.68, respectively. For intact plant, the highest accumulation of saponins was recorded in leaves which represented the second highest level of saponins accumulation. The average of saponins intensity means and medians in these leaves were 60.33 and 59.54, respectively as shown in Table (1) and Figure (4). Table (1) and Figure (3) are put at the end of the research.

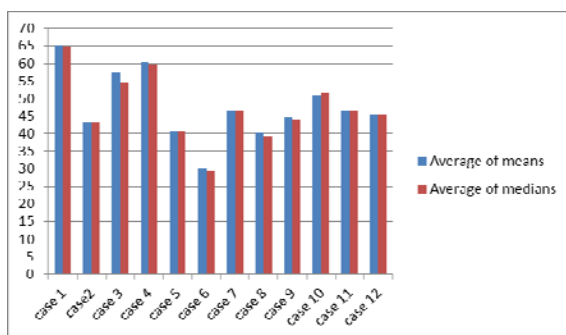


Figure 4: Averages of Means and Medians at Each Class

#### 4. CONCLUSIONS

In this research, we have presented an aid computerized system to localize saponins accumulation and evaluate the intensity of these compounds in intact plant of *Y. gloriosa* Variegata and their *in vitro* cultured tissues. The first part of the system concerned with the treatment of the problems of fluorescence microscopy, which constituted a major obstacle for the biologists despite its great scientific importance. The use of image processing techniques is a key step to ensure that the right decision is taken by the biologist. Gamma and linear stretching are first carried out to adjust contrast in the acquired images. Our analysis shows that a correction is needed to highlight the fluorescent sites in low-light imaging. It is clear to observe that the proposed model gave good results for totally isolation of saponins accumulation sites. The desired result is highly affected by the correct selection of filling region. The region that forms the ratio (0.03%) of the image area gave the best possible result according to the selected dataset. The other part of the system concerned with performing a statistical analysis and applying this analysis on the biological experiment. The results showed the high ability of the system to determine intensity of saponins in the different parts of the plant and the highest level of saponins accumulation is found in callus treated with TDZ in a combination with BA and NAA after 3 weeks of culture. The variation in saponins accumulation was attributed to the type of growth regulators, initiation period, and plant part. These results and conclusions were compatible with the results obtained by a biology researcher. From those data, we were able to point out some strength in statistical significance.

This work can be extended to perform on other important compounds in *Y. gloriosa* Variegata. In addition, the system can be extended to include other/more statistical analysis. On the other hand, it can be also used in any biological experiments that require using fluorescence microscopy.

#### 6. ACKNOWLEDGEMENTS

The authors would like to acknowledge the University of Baghdad to support its teaching staff and would like to acknowledge Dr. Ansam G. to provide us with the dataset which was used in this research.



## REFERENCES

- [1] C. Kervrann, C. O. Sorzano, S. T. Acton, J. Olivo-Marin, and Unser M, "A guided tour of selected image processing and analysis methods for fluorescence and electron microscopy," *IEEE J Sel Top Signal Process, IEEE Signal Processing Society*, Vol. 10, Issue 1, 2016 Feb, pp. 6-30. Available from: hal-01246375 DOI: [10.1109/JSTSP.2015.2505402](https://doi.org/10.1109/JSTSP.2015.2505402).
- [2] A. C. Croce, and G. Bottiroli, "Autofluorescence Spectroscopy and Imaging: A Tool for Biomedical Research and Diagnosis," *Eur J Histochem.*, Vol. 58, Issue 4, 2014 Oct 22, pp. 2461. Available from: DOI: 10.4081/ejh.2014.2461.
- [3] P. Bankhead, *Analyzing Fluorescence Microscopy Images with Image J*, UK: Queen's university Belfast, 2014 May, 195p.
- [4] M. J. Sanderson, I. Smith, I. Parker, M. D. Bootman, "Chapter1: Fluorescence Microscopy," In: J. B. Parys, M. D. Bootman, D. I. Yule, and G. Bultynck, editors, *Calcium Techniques: A Laboratory Manual*, New York: Cold Spring Harbor Laboratory Press, 2014, pp. 1-24.
- [5] M. Thakur, M. F. Melzig, H. Fuchs, A. Weng, "Chemistry and pharmacology of saponins: special focus on cytotoxic properties. Botanicals: Targets and Therapy, Vol. 1, 2011, pp. 19-29.
- [6] P. M. Dewick, *Medicinal Natural Products a Biosynthetic Approach*, 2<sup>nd</sup> ed. Wiley J, Sons. Ltd, Electric ISBN: 0470846275, 2002, pp. 515.
- [7] X. Du, and S. Dua, "Segmentation of fluorescence microscopy cell images using unsupervised mining," *Open Med Inform J.*, Vol. 4, 2010 May 28, pp. 41-49. Available from: DOI: 10.2174/1874431101004020041.
- [8] P. Matula, "Image Processing in Fluorescence Microscopy and its Utilization in Cell Biology Experiments," Brno: Masaryk University; 2012 Apr 12.
- [9] Y. H. Chang, H. Yokota, K. Abe, C. C. Li, and M. D. Tsai, "Fluorescence Microscopy Image Processing and Visualization for Analyzing Cell Kinematics, Proliferation and Attachment in Mouse Embryonic Stem Cell Culture," In: O'Conner L, editor, *Proceedings of the 16th International Conference on Bioinformatics and Bioengineering (BIBE), IEEE*, 2016 31 Oct - 2 Nov, Taichung, Taiwan: IEEE, 2016 Dec 19. pp. 222 – 229. Available from: DOI: 10.1109/BIBE.2016.44.
- [10] E. R. Davies, *Computer and Machine Vision: Theory, Algorithms and Practicalities*, 4<sup>th</sup> ed., USA: Academic Press (Elsevier), 2012 Mar 05, 912p.
- [11] C. O. Casado, *Image contrast enhancement methods* [Bachelor of Telecommunications Degree]. [Sofia]: Technical University, 2010.
- [12] E. R. Davies, *Machine Vision: Theory, Algorithms and Practicalities*, 3<sup>th</sup> ed., In: D. E. Penrose DE, editor, USA: Elsevier, 2005. 934p.
- [13] A. Siebert, "Retrieval of gamma corrected images," *Pattern Recognit Lett.*, Vol. 22, Issue 2, 2001 Feb, pp. 249-256.
- [14] P. Sukanya, and V. R. Shelke, "An experimental study of the performance of histogram equalization for image enhancement," *International Journal of Computer Sciences and Engineering (IJCSSE)*, Vol. 4, Issue 2, 2016 Apr, pp. 39-42.
- [15] V. Kumar, and P. Gupta, "Importance of Statistical Measures in Digital Image Processing," *International Journal of Emerging Technology and Advanced Engineering (IJETA)*, Vol. 2, Issue 8, 2012 Aug, pp. 56-62.
- [16] A. G. Abdul-Haleem, "A Comparative Anatomical Study for the Accumulation of Saponins in Intact Plant and its Tissue Cultures of *Yucca gloriosa* Variegata," PhD [Dissertation], Baghdad: University of Baghdad, 2015.

Table 1: The Means and Medians of Saponins Accumulation in Intact Plant of *Y. gloriosa Variegata* and Their *in Vitro* Cultured Tissues.

Cases	Type of the class	Class Id	Sample id	Mean of saponins intensity	Median of saponins intensity
Case 1	The accumulation of saponins in <i>Y. gloriosa Variegata</i> callus treated with a combination of 0.1 mg/L BA, 1.5 mg/L NAA and <b>1.0 mg/L TDZ after 3 weeks</b>	0	0	64.00	63.73
			1	64.00	61.66
			2	67.00	68.65
			<b>Average</b>	65.00	64.68
Case 2	The accumulation of saponins in <i>Y. gloriosa Variegata</i> callus treated with a combination of 0.1 mg/L BA, 1.5 mg/L NAA and <b>1.0 mg/L TDZ after 8 weeks.</b>	1	0	41.00	40.27
			1	44.00	44.58
			2	45.00	45.45
			<b>Average</b>	43.33	43.43
Case 3	The accumulation of saponins in <i>Y. gloriosa Variegata</i> vegetative <b>root in intact plant</b>	2	0	54.00	52.70
			1	56.00	55.71
			2	63.00	55.11
			<b>Average</b>	57.67	54.50
Case 4	The accumulation of saponins in mesophyll cells and bundle sheath cells of <b>intact plant leaf</b>	3	0	55.00	56.61
			1	63.00	61.30
			2	63.00	60.73
			<b>Average</b>	60.33	59.54
Case 5	The accumulation of saponins in <b>direct regenerated leaf</b> from <i>in vitro</i> culture	4	0	38.00	37.89
			1	42.00	41.85
			2	42.00	41.85
			<b>Average</b>	40.67	40.53
Case 6	The accumulation of saponins in epidermal cells and in cortical cells of <b>direct regenerated shoot</b> from <i>in vitro</i> culture	5	0	27.00	27.53
			1	29.00	28.69
			2	34.00	33.57
			<b>Average</b>	30.00	29.93
Case 7	The accumulation of saponins in cells of <b>indirect regenerated rhizome</b>	6	0	41.00	40.86
			1	49.00	48.39
			2	50.00	49.92
			<b>Average</b>	46.67	46.39
Case 8	The accumulation of saponins in cells of <b>indirect regenerated leaf</b>	7	0	35.00	34.63
			1	39.00	38.80
			2	47.00	44.39
			<b>Average</b>	40.33	39.27
Case 9	The accumulation of saponins in callus induced on <b>bud</b> explants treated with <b>0.1 mg/l BA and 1.5 mg/l NAA</b> after <b>6 weeks</b>	8	0	35.00	33.68
			1	49.00	46.56
			2	50.00	51.51
			<b>Average</b>	44.67	43.91
Case 10	The accumulation of saponins in callus induced on <b>bud</b> explants treated with <b>0.2 mg/l BA and 1.5 mg/l NAA</b> after <b>6 weeks</b>	9	0	48.00	47.13
			1	49.00	50.12
			2	56.00	57.44
			<b>Average</b>	51.00	51.56
Case 11	The accumulation of saponins in callus induced on <b>bud</b> explants treated with <b>0.2 mg/l BA and 1.5 mg/l NAA</b> after <b>8 weeks</b>	10	0	44.00	44.03
			1	46.00	46.57
			2	50.00	48.39
			<b>Average</b>	46.67	46.33

Case 12	The accumulation of saponins in callus induced on bud explants treated with <b>0.2 mg/l BA and 2.5 mg/l NAA</b> after 6 weeks	11	0	36.00	34.74
			1	49.00	47.70
			2	51.00	53.32
			<b>Average</b>	45.33	45.25

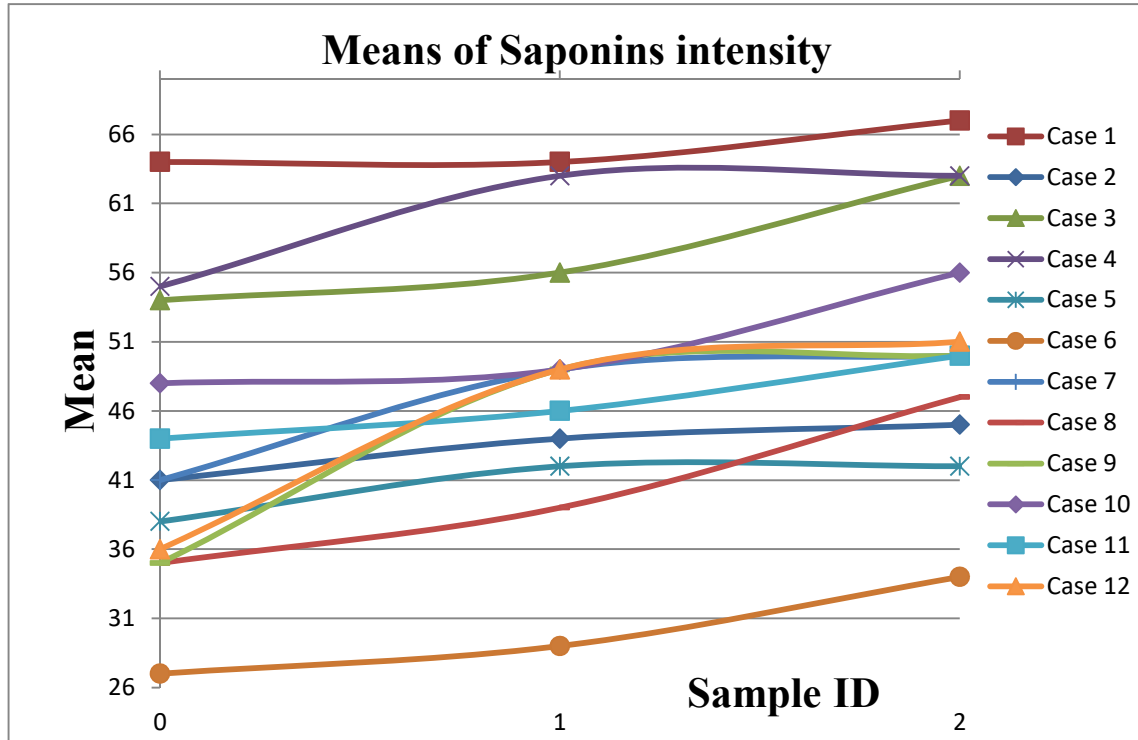


Figure 3: Mean Values of Saponins Intensity at Each Sample.