

Wound Healing and Antioxidant Activities of *Swietenia Macrophylla* Seed and *Syzygium Aromaticum* Bud

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ABSTRACT

This study analysed the wound healing and antioxidant activities of *Swietenia macrophylla* seed and *Syzygium aromaticum* bud. The antioxidant activity of *Swietenia macrophylla* seed and *Syzygium aromaticum* bud were determined using diphenyl-1-picrylhydrazyl (DPPH) scavenging assay. The cytotoxicity activity using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay against human skin fibroblast 1184 cells was used to determine the relative cell viability of the plant extract meanwhile the wound healing activity was determined by analysing the cell migration using the scratch assay. The highest percentage of antioxidant activity was shown by *Syzygium aromaticum* bud at $79.28\% \pm 0.04$ compared to $22.50\% \pm 0.00$ by *Swietenia macrophylla* seed. The highest percentage of relative cell viability for *Swietenia macrophylla* seed oil was observed at the concentration of 0.01 ppm while *Syzygium aromaticum* bud at 10 ppm. The highest percentage of wound closure observed from the mixture of 0.1 ppm *Swietenia macrophylla* seed and 10 ppm *Syzygium aromaticum* bud at 1:1 volume ratio showed the recovery percentage of $44.60\% \pm 8.42$ at 48 hours after treatment. Meanwhile, the wound closure of *Swietenia macrophylla* seed and *Syzygium aromaticum* bud were only $35.46\% \pm 7.34$ and $39.49\% \pm 6.84$ respectively. The finding indicates that the mixture of *Swietenia macrophylla* seed and *Syzygium aromaticum* bud able to treat wounds better compared to the sole usage of the plant extracts.

Keywords: *Swietenia macrophylla*, *Syzygium aromaticum*, antioxidant, cytotoxicity, wound healing

1. INTRODUCTION

The wound is an any damage or break of the skin from the underlying tissue. Over the past twenty years, the incidence of people suffering from chronic wounds has increased to more than 8.2 million [1]. Chronic wounds decrease the quality of life, leading to high morbidity and mortality rates. Wound healing is a complex, highly regulated, and self-repairing process. This process is critical in maintaining the barrier function of the skin. The wound healing process generally consists of four main phases, which are hemostasis, inflammatory, proliferative, and remodeling. Since ancient time, human has used numerous beneficial plants as the source of medical essential to treat various diseases and wounds. Nowadays, medicinal plants have become a key ingredient to produce cosmeceuticals and nutraceuticals products [2].

Swietenia macrophylla, locally known as 'tunjuk langit' in Malaysia, is one of the three species of *Swietenia* tree. The traditional remedy used the extract of the seeds and other parts of the tree to improve blood circulation, reduce blood sugar level, blood pressure and to treat various diseases such as cough, premature ejaculation, and migraine [3]. Previous

studies on *Swietenia macrophylla* seeds have reported various biological activities such as anti-inflammatory, anticancer, and antitumor [4], wound healing [5,6] as well as antidiabetic [7]. Linoleic acid is one of the major compounds in *Swietenia macrophylla* seeds oil (37.5 - 39.21%), plays a significant role in improving heart health, insulin sensitivity, and blood pressure [8]. *Syzygium aromaticum*, known as clove, is an aromatic flower bud and common spice used in culinary all over the world for centuries. The essential oil of *Syzygium aromaticum* is traditionally used in aromatherapy and as an anodyne for toothache. *Syzygium aromaticum* oil possesses biological activities such as antioxidant, antiviral, antifungal, anti-inflammatory, and anesthetic [9]. Eugenol is the primary constituent found in *Syzygium aromaticum* essential oil (88.58%), responsible for its aroma, and commonly used as antiseptic and anesthetic in dentistry [10].

2. EXPERIMENTAL

2.1. Chemicals

Analytical grade methanol, 80% ethanol, 2,2-diphenyl-1-picrylhydrazyl, Dulbecco's Modified Eagle Medium (DMEM contains D-Glucose, L-Glutamine, and Sodium Pyruvate), phosphate buffer salines, trypsin-EDTA (0.25% trypsin, EDTA 4Na), fetal bovine serum, Tween® 80, and dimethyl sulfoxide (DMSO) are purchased from Sigma-Aldrich.

2.2. Plant Material

The *Swietenia macrophylla* seed and *Syzygium aromaticum* bud were purchased from a local herbal supplier in Bangi, Selangor. Both samples were extracted using supercritical carbon dioxide extraction process and were kept in a tightly sealed storage container and stored in the freezer at -20 °C.

2.3. Antioxidant Activity

10 mg of plant extract was dissolved in 10 ml of methanol with final concentration of 1mg/ml. The solution was diluted into the concentration of 0.001 ppm to 1000 ppm. Then, 100 µL of the extract solutions were mixed with 3ml of 6x10⁻⁵ M methanolic solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). Methanolic solution of DPPH was used as a negative control, while methanol is used as a blank. The test samples were incubated in the dark for 30 minutes at room temperature. The absorbance was measured using a UV-Vis spectrometer (Model UV-160, Shimadzu, Japan) at 517nm.

The free radical scavenging activity was calculated using the equation [11]:

$$\text{Antioxidant activity(Inhibition)\%} = \frac{[A_{\text{control}} - A_{\text{sample}}]}{A_{\text{control}}} \times 100$$

Where A_{control} is the absorbance of the control and A_{sample} is the absorbance of the sample.

2.4. MTT Assay

The relative cell viability was performed by using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) to determine the suitable range of concentration of extracts tested on human skin fibroblast (HSF 1184) cell. The change of colour indicates the cell viability as the yellow tetrazole of MTT will be reduced to purple formazan in living cell. The test samples were prepared by dissolving the plant extracts in Dulbecco's Modified Eagle Medium

(DMEM) at different concentrations (0.001, 0.01, 1, 10, 100, and 1000 ppm). Untreated DMEM was used as a negative control, while ascorbic acid was used as a positive control. The HSF 1184 cell suspension was plated in 96-well plate at the density of 2x10⁵ cells/well, and incubated in the humidified atmosphere for 24 hours. After 24 hours incubation, 100µL of DMEM was mixed with 20µL MTT solution (5 mg/ml dissolved in PBS) and incubated again at 37°C for 4 hours. After that, the MTT solution of all samples were removed and replaced with 200µL DMSO for 15 minutes to allow the dissolution of the purple MTT formazan crystal. The absorbance was measured at 540 nm using ELIZA plate reader (BIOTEK, ELx-808).

The percentage of relative cell viability was calculated using following equation [12]:

$$\text{Relative cell viability (\%)} = \frac{\text{Mean absorbance of experiment cells}}{\text{Mean absorbance of control cells}} \times 100\%$$

For assessment of cell viability in HSF1184 cell exposed to the plant extracts tested, the doseresponse curves were plotted using a non-linear regression and the IC50 values were calculated with GraphPad Prism 8 software.

2.5. Scratch Assay

The human skin fibroblast (HSF 1184) cell concentration was seeded into 6-well plate tissue culture dishes by the volume of 2 ml per well. The diluted extracts were filtered using a cellulose acetate membrane to ensure the sterility of the mixture. A linear wound is created by using a 200 µL plastic pipette tip in monolayer cells. The cells were washed to remove any cellular debris by using PBS. After that, 2 ml of DMEM (negative control), ascorbic acid (positive control), and the diluted extracts which consisted of 0.01 ppm for *Swietenia macrophylla*, 10 ppm for *Syzygium aromaticum*, and the combination of both extracts at volume ration of 1:1 are added to the scratched cells and incubated for 48 hours at 37°C with 5% CO₂. Three representative images from each well were photographed at 0, 12, 24, and 48 hours to measure the wound closure.

The images were analyzed with NIH ImageJ software. The percentage of wound closure determined using the formula [13]:

$$\text{Wound closure (\%)} = \frac{[W_{t=0h}] - [W_{t=48h}]}{[W_{t=0h}]} \times 100\%$$

Where, W_{t=0h} = Width of wound at 0 hours (pixels) and W_{t=48h} = Width of wound at 48 hours (pixels).

3. RESULTS AND DISCUSSION

3.1. Antioxidant Activity

The antioxidant activity of *Swietenia macrophylla* seed and *Syzygium aromaticum* bud were shown by the percentage of scavenged free radicals, as shown in **Figure 1**. The antioxidant activity increases with the increment of the plant extract concentration. The highest percentage of antioxidant activity for *Swietenia macrophylla* seed was $22.50\% \pm 0.00$ and *Syzygium aromaticum* bud was $79.28\% \pm 0.04$ observed at the concentration of 1,000 ppm. The antioxidant property from a plant could stimulate dermal

fibroblast and keratinocytes, leading to the increase of cells proliferation, barrier formation, and formation of extracellular matrix proteins [14]. A successful treatment and management of wounds depended on the overall role of antioxidant during the process. In the present study, the scavenging of free radicals activity from the plant extracts studied could be considered as one of the possible mechanism that could contribute to its wound healing activity due its attributed in the improvement of collagen deposition [15]. According to Ben Ayed, free radical scavenging properties could attribute in the wound healing activities by reducing inflammation and improve the tissue formation [16].

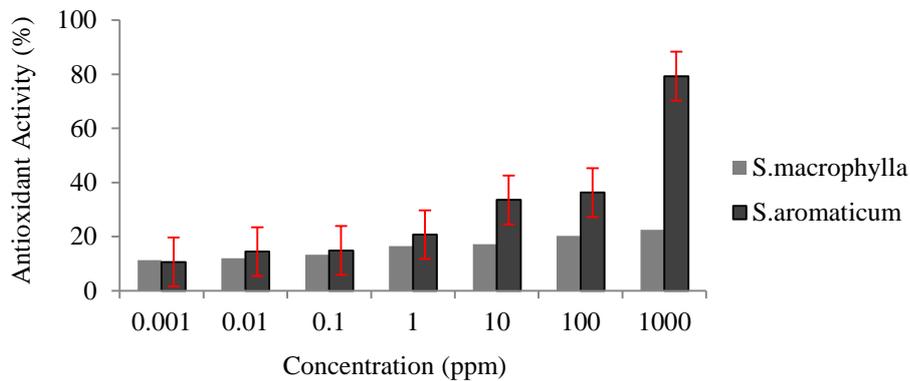


Figure 1 The percentage of antioxidant activity of extracts at different concentrations (ppm).

3.2. MTT Assay

Every medicinal plant shown different mechanism for its toxicity which affecting the cell viability such as cell membranes destruction, prevention of protein synthesis, irreversible binding on receptors, and enzymatic reactions [17]. MTT assay is one of the in vitro evaluation methods to determine the cytotoxicity level of any plant extract. This screening can help in identifying the appropriate concentration of the extract to be used on human fibroblast cells. **Figure 2** shows the percentage of relative viability of HSF 1184 cell after 24 hours treatment with plant extracts at different concentration from 0.001 ppm to 1000 ppm. The finding in MTT assay shows that the best concentration on the HSF 1184 cell was at the concentration of 0.01 ppm for *Swietenia macrophylla* seed ($108.50 \pm 6.54\%$ cell viability) and 10 ppm for *Syzygium aromaticum* bud ($113.02 \pm 7.82\%$ cell viability). At 1000 ppm, both essential oils shows strong cytotoxicity level on HSF 1184 cell at the relative

cell viability percentage of $5.73\% \pm 0.53$ for *Swietenia macrophylla* seed and $2.63\% \pm 0.17$ for *Syzygium aromaticum* bud. The finding indicates that increasing plant extract concentration may results in a higher antioxidant activity but on certain extend a high concentration of the plant extract can leads to the decreasing of cell proliferation and hence damage the cell [18]. **Figure 3** shows the half maximal inhibitory concentration (IC_{50}) of *Swietenia macrophylla* seed and *Syzygium aromaticum* bud. The IC_{50} for *Swietenia macrophylla* was 24 ppm, while *Syzygium aromaticum* was 272.27 ppm. IC_{50} determine the extract concentration to inhibit 50% inhibition of the viability on the HSF 1184 cell. The result shown *Swietenia macrophylla* seed had low toxicity compared to *Syzygium aromaticum* bud through its relative cell viability on the HSF 1184 cell at the lowest concentration.

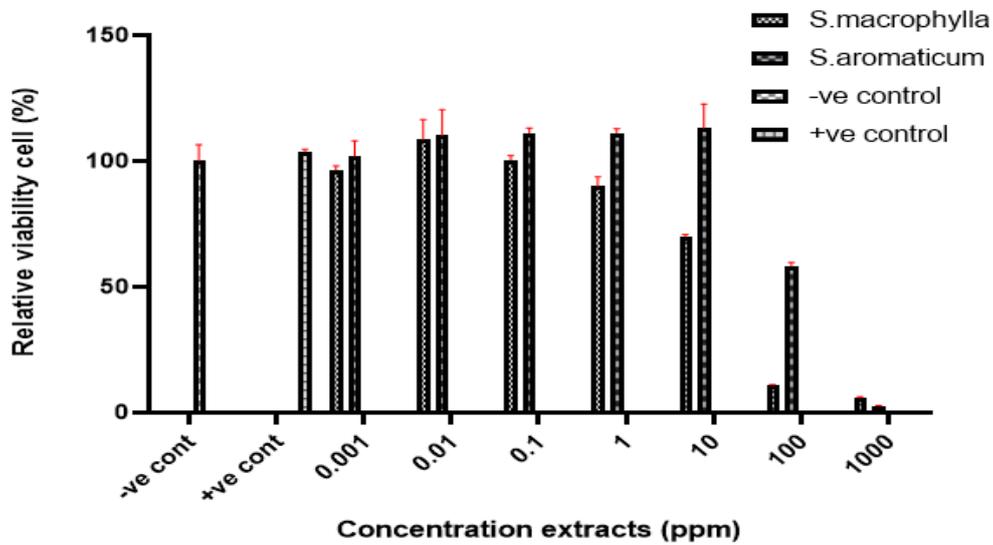


Figure 2 Effect of *Swietenia macrophylla* seed and *Syzygium aromaticum* bud on human skin fibroblast (HSF 1184) cell viability evaluated at 24 hours after treatment using MTT assay at 570 nm.

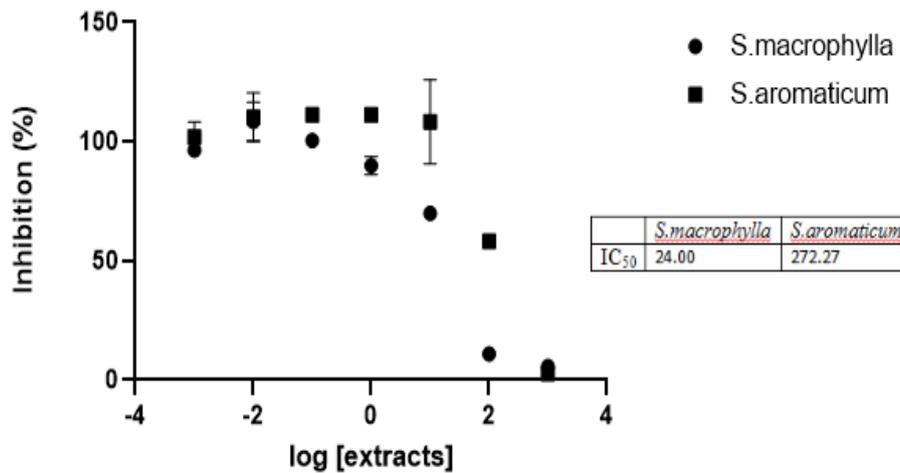


Figure 3 The IC₅₀ determination of *Swietenia macrophylla* seed and *Syzygium aromaticum* bud on MTT assay.

3.3. Scratch Assay

Scratch assay is a straightforward, cost-effective, and well-developed method to study the coordinated cell migration in vitro. This method involved in creating gap, called a 'scratch', by physical exclusion or by removing the cells from the area on a confluent cell monolayer, capturing images on regular interval to observe the cell migration in

closing the scratch, and quantifying the images comparison to determine the closure rate [19]. In this study, the ability of the *Swietenia macrophylla*, *Syzygium aromaticum*, and their mixture to induce HSF1184 cell migration into the wound area were evaluated by comparing and quantifying the wound width at 0, 12, 24, and 48 hours to determine the rate of cell migration. The plant extracts mixtures were consisted of 0.01 ppm *Swietenia macrophylla* seed and 10 ppm *Syzygium aromaticum* bud mixed at 1:1 volume ratio.

Figure 4 shows that the highest percentage of wound closure rate was shown by the mixture at $44.60\% \pm 8.42$ while the lowest percentage of wound closure was shown by *Swietenia macrophylla* seed at $35.46\% \pm 7.34$. Moreover, *Swietenia macrophylla* seed, *Syzygium aromaticum* bud, and their mixture shown higher wound closure rate compare to the positive control. This result indicates that *Swietenia macrophylla* seed and *Syzygium aromaticum* bud were able to stimulate the human fibroblast

cell (HSF 1184), resulting in the effective migration within the wounded area. Figure 5 shown the in vitro wound healing images of HSF 1184 cells in different culture mediums. The HSF 1184 cells that have migrated to the wounded area will proliferate and produce the matrix protein, thus aiding in the new construction of Extra Cellular Matrix (ECM) and lead to the further repair process.

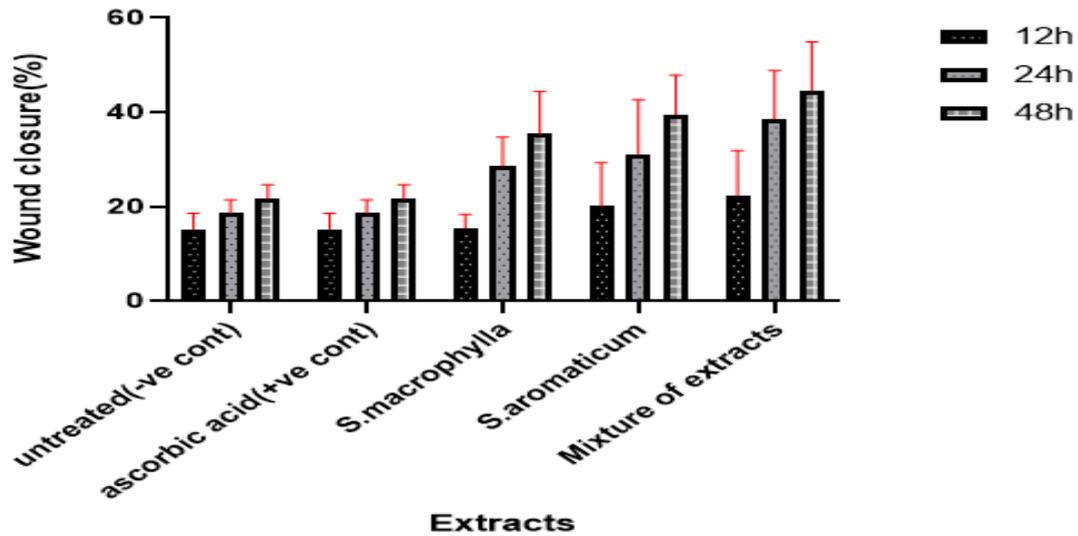


Figure 4 The wound closure rates of 0.01 ppm *Swietenia macrophylla*, 10 ppm *Syzygium aromaticum*, the mixture, negative control (DMEM +10% FBS +1% antibiotic mixture), and positive control (ascorbic acid).

4. CONCLUSION

This study found that *Syzygium aromaticum* bud has a higher antioxidant activity compare to *Swietenia macrophylla* seed. However, the cytotoxicity study shows that both plant extracts are toxic toward HSF 1184 cell at the concentration above 10 ppm hence the usage of the plant extracts should be below the mentioned concentration. The IC_{50} concentration indicates that *Swietenia macrophylla* seed was less toxic toward HSF 1184 cell compared to *Syzygium aromaticum* bud. The mixture of 0.01 ppm of *Swietenia macrophylla* seed and 10 ppm of *Syzygium aromaticum* bud at 1:1 volume ratio have the highest ability to stimulate the HSF 1184 cells migration and the presence of plant extracts were able to effectively increase the wound closure rate of HSF 1184 cell compared to the positive control.

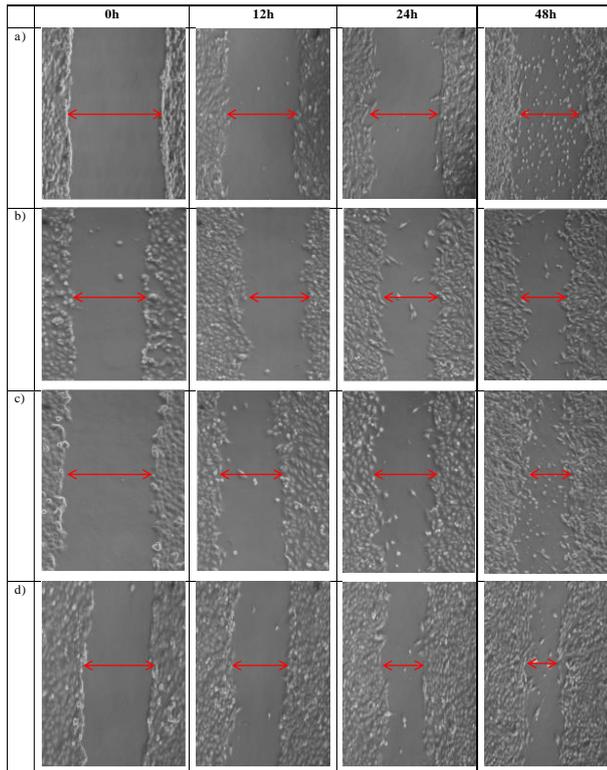


Figure 5 Representative images of *in vitro* wound healing of human fibroblast cells (HSF 1184) cultured in (a)negative control (Complete DMEM) (b) positive control (ascorbic acid) (c) *Swietenia macrophylla* extract oil at 0.01 ppm (d) *Syzygium aromaticum* extract oil at 10 ppm (e)Mixture of oil extracts. The headed arrow indicating width of the scratch (W).

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REFERENCES

[1] Sen CK. Human Wounds and Its Burden: An Updated Compendium of Estimates. *Advance Wound Care (New Rochelle)*. 2019; 8 (2): 39-48. doi:10.1089/wound.2019.0946

[2] Belwal T, Ezzat SM, Rastrelli L, Bhatt ID, Daglia M, Baldi A. A critical analysis of extraction techniques used for botanicals: Trends, priorities, industrial uses, and optimization strategies. *Trends Anal Chem*. 2018; 100: 82–102. doi: 10.1016/j.trac.2017.12.018

[3] Moghadamtousi SZ, Goh BH, Chan CK, Shabab T, Kadir HA. Biological activities and phytochemicals of *Swietenia macrophylla* king. *Molecules*. 2013;18 (9) : 10465-10483. doi: 10.3390/molecules180910465

[4] Sayyad M, Tiang N, Kumari Y, Goh BH, Jaiswal Y, Rosli R. Acute toxicity profiling of the ethyl acetate fraction of *Swietenia macrophylla* seeds and in-vitro neuroprotection studies. *Saudi Pharm J*. 2017; 25 (2) : 196-205.

[5] Hartati H, Mohd-Nasir H, Md Salleh L, Idris IS, Abd Aziz A. Evaluation of *Swietenia mahagoni* Jacq seed extracts in promoting wound healing properties. *Malaysian J Fundam Appl Sci*. 2018;14(4):432-436.

[6] Hartati, Liza Md Salleh, Irma Suryani Idris, Andi Asmawati Azis. Wound Healing Properties of Swietenia Mahagoni Seed Extracted Using Scco2: An In Vitro Study. *J Teknol*. 2019;81(1):119–23.

[7] Md Norodin NS, Md Salleh L, Yusof N, Mustapha NM, Kamarulzaman F, Zahari MAM. Inhibitory effects of *swietenia mahagoni* seeds extract on A-glucosidase and A-amylase. *Int J Eng Trans B Appl*. 2018;31(8):1308-1317.

[8] Ahmad Mustafa Masoud Eid, Nagib Ali Elmarzugi, and Hesham Ali El-enshasy. A Review on the Phytopharmacological Effect of *Swietenia Macrophylla*. *International Journal of Pharmacy and Pharmaceutical Sciences*. 2013; 5(3): 47–53.

[9] Mbaveng AT, Kuete V. *Syzygium aromaticum* - Medicinal Spices and Vegetables from Africa. Elsevier Inc. 2017;611–625.

[10] Kamel Chaieb, Hafedh Hajlaoui, Tarek Zmantar, Amel Ben Kahla-Nakbi, Mahmoud Rouabhia, Kacem Mahdouani, Amina Bakhrouf. The chemical composition and biological activity of clove essential oil, *Eugenia caryophyllata* (*Syzygium aromaticum* L. Myrtaceae): a short review. *Phytotherapy Research*. 2007; 21(6):501-6.

[11] Tushar Kanti Bera, Kausik Chatterjee, Debidas Ghosh. In-vitro antioxidant properties of the hydro-methanol extract of the seeds of *Swietenia mahagoni* (L.) Jacq. *Biomarkers and Genomic Medicine*. 2015; 7(1), 18-24.

[12] Raheem Mohssin Shadhan Siti Pauliena Mohd Bohari. Effects of *Hibiscus sabdariffa* Linn. fruit extracts on α -glucosidase enzyme, glucose diffusion and wound healing activities. *Asian Pacific Journal of Tropical Biomedicine*. 2017; 7(5), 466-472.

[13] Nur Syukriah Ab. Rahman, Liza Md. Salleh, Harisun Yaakob, Fadzillah Adibah Abdul Majid. Cells migration potential of *Quercus infectoria* aqueous extract evidenced in human skin fibroblast scratch assay method. *Regenerative Research*. 2013; 2(2), 43-47.

[14] Süntar I, Akkol EK, Nahar L, Sarker SD. Review Article Wound healing and antioxidant properties : do they coexist in plants. *Free Radicals Antioxidants*. 2012;2(2):1-7.

[15] David Emery Tsala, Nnanga Nga, Bella Ndzana Martin Thiery, Mballa Therese Bienvenue, and Dimo Theophile. Evaluation of the antioxidant activity and the healing action of the ethanol extract of *Calotropis procera* bark against surgical wounds. *J Intercult Ethnopharmacol*. 2014;4(1):64-69.

[16] Hanen Ben Ayed, Sana Bardaa, Dorsaf Moalla, Mourad Jridi, Hana Maalej, Zouheir Sahnoun, Tarak Rebai, Phillipe Jacques, Moncef Nasri, Noomen Hmidet. Wound healing and in vitro antioxidant activities of lipopeptides mixture produced by *Bacillus mojavensis* A21. *Process Biochemistry*. 2015;50(6):1023-1030.

[17] Ishiyama M, Tominaga H, Shiga M, Sasamoto K, Okhura Y, Ueno KA. Combined assay of cell viability and in vitro cytotoxicity with a highly water-soluble tetrazolium salt, neutral red and crystal violet. *Biological & Pharmaceutical Bulletin*. 1996; 19(11):1518-1520.

[18] Hartati, Liza Md Salleh, Azila Abd. Aziz, Mohd. Azizi Che Yunus. The Effect of Supercritical Fluid Extraction Parameters on the *Swietenia Mahagoni* Seed Oil Extraction and its Cytotoxic Properties. 2014;65(5):51-53.

[19] Chun-Chi Liang, Ann Y Park, Jun-Lin Gua. In vitro scratch assay: a convenient and inexpensive method for analysis of cell migration in vitro. *Nature Protocols*. 2007;2, 329-333.