journal of natural science review

Vol. 3, No. 1, 2025
https://kujnsr.com
e-ISSN: 3006-7804

The Impact of Russian Knapweed Extracts on Open Wound Healing Process in Rabbits

Ghulam Haidar Olfat[1], Emal Habibi [2], Mohammad Sangary [3], Amanullah Aziz [4], Said Ashraf Hashemi [5], Nick Mohammad Omari [6]

[1,4,5,6] Kabul University, Department of Clinic, Faculty of Veterinary Science, Kabul, Afghanistan
[2] Kabul University, Department of Animal Production, Faculty of Veterinary Science, Kabul, Afghanistan
[3] Kabul University, Department of Paraclinic, Faculty of Veterinary Science, Kabul, Afghanistan
✉E-mail: gh.olfat123@gmail.com (corresponding author)

ABSTRACT

Skin is the body's outermost layer; therefore, it is more at risk and is injured by many factors such as surgery, illness, and burns. Numerous drugs are used to heal these wounds, each of which has several drawbacks, limitations, and side effects. So, it is important to prepare a substance that is accessible, cheap, without limitations and defects, with or without low side effects. Therefore, we decided to conduct a study to investigate the effects of extracts of Russian knapweed on open wound healing in rabbits. This research was conducted on 18 rabbits in the Faculty of Veterinary Sciences of Kabul University. Russian Knapweed root and stem extract were extracted using the decoction method. Then, a deep open wound was created in the animal's skin by a scalpel blade, and extracts were applied. The rabbits were randomly divided into the three (control, root, stem) groups. Results obtained from this research show that the vital signs of rabbits during the research were typical also on the fourth day; the root and stem groups had the highest healing rate, respectively. There is a significant difference compared to the control group, $P < 0.05$. On days 7th, 10th, and 13^{th,} the stem group had the highest healing rate, but the difference between this group and the control and root groups was insignificant $P>0.05$. Generally, the results of this study show that the Russian Knapweed root and stem extract obtained by the decoction method did not have a good effect on wound healing. However, on the fourth day of wound evaluation, more than 50% of wounds in root and stem groups were healed.

ARTICLE INFO

Article history:

Received: December 25, 2024
Revised: March 01, 2025
Accepted: March 20, 2025

Keywords:

Extract; Healing; Rabbits; Russian knapweed; Wound

To cite this article: Olfat, G. H., Habibi , E., Sangary , M., Aziz , A., Hashemi , S. A., & Omari , N. M. The Impact of Russian Knapweed Extracts on Open Wound Healing Process in Rabbits. *Journal of Natural Science Review*, *3*(1), 55–64. DOI: https://doi.org/10.62810/jnsr.v3i1.177

Link to this article: https://kujnsr.com/JNSR/article/view/177

INTRODUCTION

The integumentary system is the outermost part of the body (Allen & Harper, 2011). This part includes the skin with its attached structures, such as hair and its glands, horns, hoofs, claws, and other epithelial coverings of the body (Fails & Magee, 2018). The skin is also called an integument or cutaneous membrane (Allen & Harper, 2011). The skin protects the body

against pathogens and chemicals, minimizes loss or entry of water, prevents the harmful effects of sunlight, and produces Vitamin D. Sensory receptors in the skin provide information about the external environment and help the skin regulate the body temperature in response to environmental changes. The subcutaneous tissue is a secondary line of defense against pathogens, a site of fat storage, and the other metabolic functions of adipose tissue (Scanlon & Sanders, 2007). The skin has two main parts: the superficial epidermis and the deep dermis. The epidermis (epi - above), the outer layer of the skin, is composed of epithelial tissue. The dermis is a layer of connective tissue attached to the epidermis by a basement membrane, providing nutrients to the avascular epidermis and connecting it to the underlying hypodermis. Hypodermis (hypo-below), or subcutaneous layer is not a part of the integumentary system, because it is located under the skin; it is studied with the skin. The hypodermis is a major storage site for adipose tissue (Allen & Harper, 2011).

As mentioned, the skin is the body's outermost layer; therefore, it is more at risk. Every day, thousands of people and animals are injured by many factors, such as surgery, illness, burns, or various physical injuries. As a result of the mentioned factors, a part of the body tissue is damaged breaks, and as a result, the nutrition system of the tissue is disturbed.

Disorders caused by physical, chemical, thermal, microbial, or immune factors in the function or structure of normal tissue are called wounds (Masson-Meyers et al., 2020). The healing process begins immediately after injury. The following four stages include this process: hemostasis (coagulation), acute inflammation, proliferation (granulation), and regeneration (maturation, contraction).

The blood vessels contract in response to the injury immediately for blood coagulation, and the platelets stick to the collagen of the damaged endothelial cells. If the platelets are not involved, additional bleeding is possible because the vessels relax quickly. After adhesion, platelets secrete vasoconstrictors to (1) maintain constriction of the damaged vessels, (2) initiate the process of thrombogenesis to plug the leak in the vessels and prevent additional bleeding, and (3) initiate blood vessel repair (Ackermann, 2012). Unless infection, trauma, or some disorder intervenes, the inflammatory (acute inflammation) phase of wound healing is fully established 24 hours after vascular injury; it can continue for 96 hours or more. Redness, swelling, heat, pain, and loss of function are the main symptoms of inflammation (Ackermann, 2012).

One of the most important functions of inflammation is the transfer of leukocytes, especially neutrophils, and monocytes, to the site of injury because leukocytes swallow and kill bacteria and destroy necrotic tissue and immune complexes. Leukocytes can defend themselves with their lysosomal enzymes. New endothelium (angiogenesis), epithelium (epithelialization), and connective tissue (fibroplasia/desmoplasia) are produced in the proliferation stage to restore the damaged tissue to its previous normal state (Ackermann, 2012).

Angiogenesis (neovascularization): At this stage, in the place of damage, endothelial cells proliferate from the edge of the cut blood vessels and form new blood vessels. The proliferating endothelial cells are initially solid buds, and within a few hours, they develop into a lumen and begin to carry blood. Soon, these blood vessels differentiate into muscular arterioles, thin-walled venules, and true capillaries. Angiogenesis is influenced by Vascular Endothelial Growth Factor (VEGF), Platelet-Derived Growth Factor (PDGF), Transforming Growth Factor-Beta (TGF-b), and Basic Fibroblast Growth Factor (BFGF). After wound formation, more energy is required for various processes such as cell proliferation, migration, and collagen production. Hence, angiogenesis is important for proper wound healing because oxygen and nutrition that produce energy are transferred through these vessels (Mohan, 2015).

Epithelialization: In this process, healthy epidermal cells migrate, proliferate from the edges of the wound, and create a new basement membrane. This process starts from the edge and continues toward the center of the wound. A new epithelium is created on the wound's surface at this stage. Generally, this process is regulated by cytokines produced by platelets, macrophages, fibroblasts, and keratinocytes (Vegad, 2008).

Fibrosis: The process of fibrosis, fibroplasia, or scar formation occurs with (1) the migration and proliferation of fibroblasts to the site of injury and (2) the deposition of Extra Cellular Matrix (ECM) by these cells. Platelet-derived growth factor, essential fibroblast growth factor, and beta-transforming growth factor are the factors that play a role in the migration of fibroblasts to the site of injury and their proliferation. Platelets, inflammatory cells, and active endothelium are the origin of these factors. One of the important cellular components of granulation tissue is macrophages. Macrophages clean the cellular debris, fibrin, and other foreign substances in the injury site and produce growth factors that cause the migration and proliferation of fibroblasts and the production of ECM. The ECM fibroblasts produce collagen (mainly type I and III), fibronectin, elastin, and proteoglycans. These facilitate cell migration and provide mechanical support for new capillaries, providing more nutrients to maintain cell metabolism. Epithelialization, granulation tissue formation, and angiogenesis co-occur during the proliferative phase (Vegad, 2008).

After completing the inflammatory and proliferative phases, the regeneration phase (remodeling, maturation, contraction) begins. In this stage, the immature connective tissue is transformed into mature connective tissue, regenerating the granulation tissue by forming extracellular collagen. At this stage, the strength of the newly formed tissue increases as the collagen fibers become closer and denser, and unnecessary cells are removed by apoptosis. One of the key components of wound healing is ECM and stem cells (fibroblasts, myofibroblasts). Myofibroblasts contract the wound, bringing the damaged and separated tissue back together (Ackermann, 2012).

Wound healing is generally completed in the four mentioned stages. However, each system has unique types of cells that affect the wound-healing process; for example, bone repair is done by callus formation, and skin repair by epithelialization (Ackermann, 2012).

Wound healing is one of humankind's most essential issues since creation. Numerous drugs are used to heal wounds, each of which has several drawbacks, limitations, and side effects. Today, the use of medicinal plants to reduce the side effects of chemical drugs for therapeutic purposes is welcomed. In traditional medicine, various herbs and natural materials are used to heal wounds because the use of herbal compounds is both cheaper and safer than other treatment methods, and it also has beneficial effects due to the presence of various medical compounds (Babaei-ghaghelestany *et al*., 2022).

People in developing countries believe that plants have antimicrobial properties. Therefore, approximately 60 to 90 percent of people use plants to treat microbial diseases. Plants contain chemical substances such as tannins, terpenoids, alkaloids, and flavonoids. Scientists are trying to make plant extracts to treat cancer and viral and microbial infections (Akhgari et al., 2022).

Drug resistance poses a significant challenge, prompting unprecedented efforts to reduce it. So, they found that drug resistance is significantly reduced when herbal compounds are used instead of pharmaceutical company drugs because plants synthesize various antimicrobial compounds to defend themselves against pathogenic microorganisms. Therefore, it can help solve the problem of antibiotic resistance in pathogenic microorganisms. Although much research has been done to identify plants' beneficial compounds due to their multiplicity and having multiple and unique compounds, more studies are needed to identify their therapeutic properties (Babaei-ghaghelestany *et al*., 2022).

Also, as mentioned above, various physical, chemical, and biological factors cause wounds in thousands of humans and animals today. Given the high incidence of this complication, it is important to prepare a substance that is accessible, cheap, without limitations and defects, and with or without low side effects. Therefore, we decided to conduct a study to investigate the effects of extracts of Russian knapweed on open wound healing in rabbits.

Acroptilon repens (Russian knapweed) belongs to the Asteraceae family. This plant is a perennial herbaceous. A. repens is native to Turkey, Central Asia, and China (Callaway & Schaffner, 2011). It has upright stems and heights between 30 and 80 cm (Koloren et al., 2008). The branches are broad. The color of this plant is pink to purple. The common name of this plant is Talkhe in Persian (Akhgari et al., 2022). A. repens grows well in areas with much water (Mangold et al., 2007). Russian knapweed can produce up to 1,200 mature seeds per shoot. Plant seeds are often transported by machinery or transportation of contaminated hay and less often by wind (Gaskin & Littlefield., 2017).

A repens has been used in traditional medicine as an emetic, antiepileptic, and anti-malaria worldwide. New studies on A. repens have shown that this plant has medicinal properties such as antimicrobial, antipyretic, fat-reducing, antioxidant, and antidiabetic effects. It has also been reported that A. repens extract has intense cytotoxic activity against

P-388 and HL-60 tumor cell lines, and A. repens has polyphenolic compounds (Dashti *et al*., 2022; Babaei-ghaghelestany *et al*., 2022). Due to the antioxidant, anti-inflammatory, and antimicrobial properties of Russian Knapweed, this plant may be effective in wound healing.

In Afghanistan, most people who keep animals live in rural areas and do not have enough access to cities. In many circumstances, when their animal becomes sick or injured, they do not have access to veterinary services (medicine) to treat them. On the other hand, the price of medicine is unsuitable for most animal owners due to their economic situation. Besides the mentioned problems, most drugs have adverse effects and can even cause severe negative effects on animal and public health. In this case, the best option is homemade drugs made from medical plants. Therefore, this study aims to investigate the effects of Russian knapweed extracts on open wound healing in rabbits.

MATERIAL & METHODS

Study Area

This experimental and mixed research was conducted on 18 Dutch rabbits in the Faculty of Veterinary Sciences of Kabul University. After purchasing from Kah Froshi market, rabbits were kept at room temperature with natural light until the end of the research. During this time, enough water and food (Alfalfa) were available to them.

Preparation of Russian Knapweed extract

Russian Knapweed plant was collected from the north of Kabul, Shekar Dara district, and transferred to Kabul University, Faculty of Veterinary Sciences. Russian Knapweed root and stem extract was obtained using the decoction method. A decoction is a water-based preparation to extract active compounds from medicinal plant materials.

After collecting, the Russian Knapweed plant was washed to remove its dust. Later, its root and stem are separated and cut into smaller pieces and heated in water for about an hour. and in the meantime, the pot contents are stirred every few minutes so that the Russian Knapweed extract is well separated from them. Then, the contents of the pot were filtered by a piece of muslin. The filtered solution was put in the pot and put on medium heat again to enhance its concentration, and in this way, Russian Knapweed root and stem extracts were obtained (Rasul, 2018).

Preparation for Wounding

Before starting the experiment, the health of the animals was confirmed using physical examination, and they were kept in the faculty laboratory for three days to get an agreement on the new environment. Then, the hairs on the back and left side of the rabbits were shaved, and the place where the wound was created was scrubbed or disinfected. After that, the animal was kept in a fixed position, and a 15mm x 15mm square was drawn in the left area, lower than the spine, using a ruler and a pen with a narrow tip. Then, two percent lidocaine was injected subcutaneously into the four corners of the square to create local anesthesia.

Then, a deep open wound was created in the skin of the animal by scalpel blade number (11). (Zaki, El-Bakry, & Fahmy, 2005).

Grouping

The rabbits were randomly divided into three groups, with six rabbits in each group.

I. Control group: no intervention was done on the wounds of this group, but they were left in a natural state.

II. Root experimental group: the wounds of this group were treated with the extract prepared from Russian Knapweed root.

III. Stem experimental group: the wounds of this group were treated with the extract prepared from Russian Knapweed stem.

After creating a wound in the animals, two times a day (morning and evening) at certain times, the mentioned extracts were applied on the wound's surface.

Wound Measurement

A ruler on the 1st, 4th, 7th, 10th, and 13th, days after surgery measured the wound surface. On the first day,, all rabbits have created a clean, open, cutaneous wound. The size of the wounds was 15mm×15mm. In addition to the mean and standard deviation, the wound healing rate has been shown as a percentage; therefore, the percentage of healing on the first day was considered (0); as the size of the wound decreases, the percentage of the healing rate increases.

Percentage calculation formula: Percentage of wound healing = (Total wound area – Present wound area) / (Total wound area) × 100

Statistical Analysis

The data were shown as percent using Percentage calculation formula and mean and standard deviation using SPSS software version 27. The Non-Parametric Kruskal Wallis statistical test was used to determine the difference between the groups, and $P<0.05$ was considered a significant level.

RESULTS

Vital Signs

The present research was conducted to investigate the effectiveness of Russian Knapweed stem and root extract in healing open wounds. The results of this research show that vital signs (temperature, heart rate, respiration rate) and mucous membrane of eyes of rabbits during the research were normal.

Wound Healing

On fourth day, the root and stem groups had the highest healing rate, respectively. There is a significant difference in wound healing rate between Root and stem groups compared to the control group, P < 0.05 (Table & Graph 1).

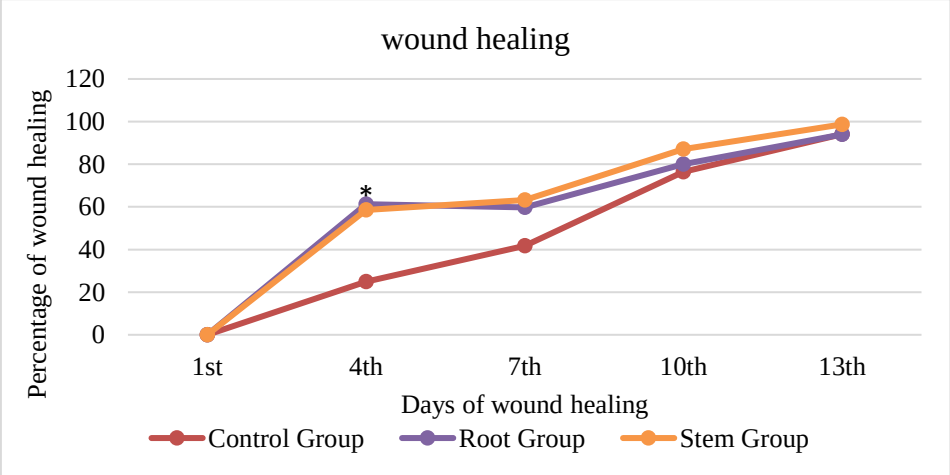
On the seventh day, the stem group had the highest healing rate, but the difference between this group and the control group was insignificant, P > 0.05. The difference between the root group and the control group was also not significant P > 0.05 (Table & Graph 1).

On the 10th day, wound healing increased in all groups, but the difference in healing between groups was insignificant P > 0.05 (Table & Graph 1).

On the 13th day, the stem group had the highest healing rate and almost completely healed. However the root and control groups had the lowest and same healing rate, although this difference between the groups was not significant statistically P > 0.05 (Table & Graph 1).

*Table 1: Wound healing size as Means± SD. *P<0.05*

Parameter	Day	Control	Root	Stem
Wound size/ mm	1	225±0	225±0	225±0
	4	168.6±21.4	87.3±17.9*	93.1±49*
	7	131.1±41.3	90.6±30.4	82.8±60.2
	10	53.1±41.7	43.8±26.3	29±34.1
	13	13.1±13.6	13.5±10.2	2.8±4.7



*Graph 1: Percentage of wound healing *P<0.05*

DISCUSSION

Natural products with anti-inflammatory, antioxidant, and antimicrobial properties are usually used for the treatment of wounds (Davoodi *et al.*, 2022). As Russian Knapweed has these properties (Babaei-ghaghelestany *et al.*, 2022; Akhgari *et al.*, 2022), we investigated its effect on wound healing in rabbits.

Natural products contain ingredients such as Tannins, flavonoids, alkaloids, saponins, vitamins, minerals, and steroids; these compounds can play an important role in wound healing (Nasa & Kumar, 2020; Ekradi *et al*., 2021; Mazhar *et al*., 2022). The effectiveness of Russian Knapweed in cutaneous wound healing is also due to its compounds, such as flavonoids and triterpenes (Quintana *et al*., 2008; Nadaf *et al*., 2013).

This study showed no significant difference between root and stem of Russian Knapweed in the studied groups on different days, but root and stem groups had significant differences with the control group on the fourth day.

The results of this study also showed that the groups of root and stem are more effective in healing cutaneous wounds in rabbits than the control group on the fourth day. This finding agrees with the statement of (Gou *et al*., 2023), who says that all plants of the Asteraceae family have effects on wound healing (Gou *et al*., 2023).

Also, the good healing effect of this plant may be due to its phenolic and flavonoid compounds because hydro-ethanol extract from *Vitis labrusca* leaves was found to advance the healing of wounds due to the total phenolic and flavonoid content (El Sherbeni, 2023). However, on the seventh day, the wounds of the root and stem group became infected and inflamed; this may be due to the presence of water in the extract. According to Abubakar & Haque (2020), water in the extract causes the growth of bacteria and molds.

CONCLUSION

The results of this study show that the Russian Knapweed root and stem extract obtained by decoction method did not have a good effect on wound healing, although on the fourth day of wound evaluation, more than 50% of wounds in root and stem groups were healed. In order to obtain more accurate effectiveness of the extract of this plant, we suggest that more research should be done taking into complete hygiene conditions so that the wounds should not be infected.

Acknowledgments

The authors thank everyone who helped us rear and feed the rabbits throughout the experiment.

Conflicts of Interest

The authors declare that there is no conflict of interest.

REFERENCES

Abubakar, A. R., & Haque, M. (2020). Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. *Journal of Pharmacy and Bioallied Sciences*, *12*(1), 1–10. https://doi.org/10.4103/jpbs.JPBS

Ackermann, M. R., (2012). Zachary, J. F., McGavin, D. M.(ed.) In Pathologic basis of veterinary disease, (5th, ed). Elsevier, Penny Rudolph, ISBN: 978-0-323-07533-6, pp.

89-135.

Akhgari, Z., Tanomand, A., Nazari, R., & Zargar, M. (2022). Biological and antibacterial features of Acroptilon repens (L .) DC. *Crescent Journal of Medical and Biological Sciences*, *9*(2), 116–122. https://doi.org/10.34172/cjmb.2022.20

Allen, C., & Harper, V. (2011). Laboratory manual for anatomy and physiology (4th, ed). John Wiley & Sons, ISBN-13 978-0470-59890-0, United States of America, pp. 83-90.

Babaei-ghaghelestany, A., Taghi, M., & Farzaneh, S. M. M. (2022). The anticancer and antibacterial properties of aqueous and methanol extracts of weeds. *Journal of Agriculture and Food Research*, *10*(2022), 100433. https://doi.org/10.1016/j.jafr.2022.100433

Callaway, R. M., & Schaffner, U. (2011). The impact of Acroptilon repens on co-occurring native plants is greater in the invader' s non-native range. *Division of Biological Sciences,* https://doi.org/10.1007/s10530-011-0145-1

Dashti, A., Shokrzadeh, M., Karami, M., & Habibi, E. (2022). Phytochemical identification, acute and subchronic oral toxicity assessments of hydroalcoholic extract of Acroptilon repens in BALB / c mice : A toxicological and mechanistic study. *Heliyon*, *8*(2022), 1–12. https://doi.org/10.1016/j.heliyon.2022.e08940

Davoodi, F., Raisi, A., Farjanikish, G., Abdollahzadeh, H., & Kamalpour, M. (2022). A Review on wound healing with Iranian medicinal plants and microbial flora in veterinary medicine. *Iranian Journal of Veterinary Surgery*, *17*(2), 146–159. https://doi.org/doi.org/10.30500/IVSA.2022.345708.1304

El Sherbeni, S.A., Negm, W. A., (2023). The wound healing effect of botanicals and pure natural substances used in in vivo models. *Inflammopharmacology*, *31*(2), 755–772. https://doi.org/10.1007/s10787-023-01157-5

Fails, A.D.,. Magee, C., (2018). Anatomy and physiology of farm animals (8th, ed). Wiley-Blackwell, ISBN 9781119239734, United States of America, pp. 273-276.

Gaskin, J. F., & Little, J. L. (2017). Invasive Russian Knapweed (Acroptilon repens) creates large patches almost entirely by Rhizomic growth. *Invasive Plant Science and Management*, *10*, 119–124. https://doi.org/10.1017/inp.2017.9

Golam Rasul, M., (2018). Conventional extraction methods used in medicinal plants, their advantages and disadvantages. *International Journal of Basic Sciences and Applied Computing*, *2*(6), 10–14.

Gou, J., Lu, Y., Xie, M., Tang, X., Chen, L., Zhao, J., Li, G., & Wang, H. (2023). Antimicrobial activity in Asterceae: The selected genera characterization and against multidrug resistance bacteria. *Heliyon*, *9*(2023), 1–24. https://doi.org/10.1016/j.heliyon.2023.e14985

Koloren, O., Uygur, S., Bozdogan, O., Uygur, F. N., & Schaffner, U. (2008). Population

density and reproductive output of Acroptilon repens L ., in turkey. *Pak. J. Bot*, *40*(6), 2259–2263.

Mangold, J. M., Poulsen, C. L., Carpinelli, M. F., Mangold, J. M., Poulsen, C. L., & Carpinelli, M. F. (2007). Revegetating Russian Knapweed (Acroptilon Repens) infestations using morphologically diverse species and seedbed preparation revegetating, *Rangeland Ecology & Management, 60*(4), 378–385. https://doi.org/http://dx.doi.org/10.2111/1551-5028(2007)60[378:RRKARI]2.0.CO;2

Masson-meyers, D. S., Andrade, T. A. M., Caetano, G. F., Guimaraes, F. R., Leite, M. N., Leite, S. N., & Frade, M. A. C. (2020). Experimental models and methods for cutaneous wound healing assessment. *International Journal of Experimental Pathology*, 1–17. https://doi.org/10.1111/iep.12346

Mazhar, M. W., Ali, H., Saif, S., Raza, A., Kousar, S., Tahir, H., & Mazhar, F. (2022). Therapeutical medicine for wound healing. *American Journal of Biomedical Science & Research*, *15*(4), 419–429. https://doi.org/10.34297/AJBSR.2022.15.002133

Mohan, H. (2015). *Textbook of PATHOLOGY* (7th ed.). Jaypee Brothers Medical Publishers (P) Ltd, New Delhi India, ISBN: 978-93-5152-369-7, pp. 116-163.

Nadaf, M., Asrabadi, M. N., Khalilabad, M. H., & Mohaddesi, B. (2013). Identification of non-polar chemical compounds Acroptilon repens growing in Iran by GC-MS. *Middle-East Journal of Scientific Research*, *17*(5), 590–592. https://doi.org/10.5829/idosi.mejsr.2013.17.05.75149

Nasa, P., & Kumar, H. (2020). Plants Havings Wound Healing Property : *A Review*. *12*(8), 1071–1075.

Quintana, N., Weir, T. L., Du, J., Broeckling, C. D., Rieder, J. P., Stermitz, F. R., Paschke, M. W., & Vivanco, J. M. (2008). Phytotoxic polyacetylenes from roots of Russian knapweed (Acroptilon repens (L.) DC.). *Phytochemistry*, *69*(2008), 2572–2578. https://doi.org/10.1016/j.phytochem.2008.07.015

Scanlon, V.C., Sanders, T., (2007). Essentials of Anatomy and Physiology (5th, ed). F. A. Davis Company, United States of America, ISBN–10: 0-8036-1546-9, pp. 88-100.

Vegad, J. L. (2008). A textbook of veterinary general pathology (2th ed). *International book distributing co,* ISBN 978-81-8189-181-5, India, pp. 105-210.

Zaki, A., El-Bakry, H., & Fahmy, A. (2005). Effect of licorice on wound healing in rabbits. *The Egyptian Journal of Hospital Medicine*, *20*, 58–65.