Hunan Daxue Xuebao/Journal of Hunan University Natural Sciences
ISSN：1674-2974 | CN 43-1061 / N

DOI 10.17605/OSF.IO/STR76
Vol: 60 | Issue: 05 | 2023
Asma Ahmed et al,. (2023)

IN VIVO WOUND CONTRACTION EFFICACY OF PHYTO- EXTRACTS FROM MEDICINAL PLANTS OF PAKISTANI ORIGIN

HAFSA WAHEED
Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Islamic Republic of Pakistan.

ASMA AHMED
Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Islamic Republic of Pakistan.
Corresponding Author Email: asma.ahmed@imbb.uol.edu.pk, asma.ahmad.aridian@gmail.com

REHANA BADAR
Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Islamic Republic of Pakistan. Department of Biological Sciences, Superior University, Lahore, Punjab, Islamic Republic of Pakistan.

SAMRA HAFEEZ
Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Islamic Republic of Pakistan.

AMINA AHMED
Department of Acute Medicine, Russell Hall Hospital, Pensnett Road, West Midlands Dudley DY1 2HQ United Kingdom.

Abstract

Wound healing is a complex process and has been the subject of intense research for a long time. Plants are natural sources which have been widely used for wound healing. In current study, polarity based extracts of Syzygium cumini (L.), AzadirachtA.indica A. Juss, Eucalyptus globulus Labill and Nicotiana tabacum (L.) has been used for their wound healing potential on excision wounds after seven, fourteen and twenty one days in albino Wistar rats (both genders, 250-300 g). Statistically analyzed results (at p≤ 0.05) showed that among all plant, after day 07, day 14 and day 21, ethanolic extract from leaves of A.indica (A.) (37.5 %, 75 %, 95 % respectively) and E. glogulus (Labill.) (70 %, 95 % and 100 % respectively) and water extract of S.cumini (L.) (42.5 %, 87.5 % and 100 % respectively) and N. tabacum (L.) (7 %, 86 % and 91 % respectively) showed significant wound contraction in female rats as compared to male rats, while n-Hexane extract of all plants have least significant effects. Current study can be used as basic study for the isolation of active compound behind it so that it could be used for the formulation of curative bandage to cure excision wounds.

INTRODUCTION

Wound healing process is a complicated biological process that consists of blood coagulation, inflammation, proliferation, and remodeling. The living body switches the damaged or injured tissue by this complex and active process. Healing gets slowed down under certain pathological conditions such as heavy blood loss, microbial infection, or diabetes. Therefore, it is important to get wound treated instantly with suitable handling. So, for the wound healing process, wound dressings are one of the most important and major materials used (Mohandas *et al*., 2015).

DOI 10.17605/OSF.IO/STR76
Vol: 60 | Issue: 05 | 2023
Asma Ahmed et al,. (2023)

Wounds are generally classified according to the underlying cause of the development of wounds. In acute wounds, there is tissue damage/injury that generally occurs through an orderly and time-reparative phase that results in the anatomical and functional integrity being restored sustainably. Acute wounds are typically caused by the cuts or surgical incisions. In closed wounds, the blood escapes from the circulatory system but stays inside the body. It becomes evident in the form of bruises. Blood leaks from the body through an open wound and bleeding is clearly noticeable. The open wound may be divided further into categories according to the source causing the wound. Incised wound is a wound with no loss of tissue and minor damage to tissue. It is caused primarily by sharp objects like a scalpel or knife. Puncture wounds are caused by an object which, like a nail or a needle, which punctures the skin. Since dirt may penetrate deep into the wound, chances of infection are common in them. Chronic wounds are wounds that have not gone through the usual healing stages and hence reach a state of pathologic inflammation. They need extended healing time (Sharma *et al.,* 2021).

Nicotiana tabacum (L.) is one of the most commonly planted crops in the world. Despite of this, it has a huge economical, agricultural and social value. It has been widely used for smoking, as well as for chewing and sniffing. There are more than 600 known *N. tabacum* (L.) species, but only 2 of them are used in human consumption. *N.tabacum* (L.) is native for South America, but it is growing all over the world, including Republic of Croatia, and the larger world producer is China (Banozic *et al.,* 2018). In biotechnology, *N.tabacum* (L.) plant is often used as model plant for producing cell culture and genetic engineering, because the *N. tabacum* (L.) life cycle from seed to seeding last only 3 months. Moreover, even the first transgenic plant is *N. tabacum* (L.) (Banozic *et al.,* 2019). The chemical composition of the *N.tabacum* (L.) leaves is changed during maturing, drying, fermenting, processing and storage, and is affected by the method of variety, cultivation, climatic area and many others (Banozic et al., 2018). During the drying process, the starch content decreases while the content of reducing sugars increase. In addition, during the fermentation process, the proportion of polyphenols and carbohydrates decreased, as well. A number of researchers have reported different groups of substances in *N. tabacum* (L.), such as alkaloids (including nicotine), polyphenols, terpenoids and essential oils (Cvetanovska et al., 2017), aroma compounds, limonene, indole, pyridine, fatty alcohols, phytosterols (Banozic *et al.,* 2019).

Eucalyptus globulus (L.) essential oils, as well as leaves, are used for therapeutic purposes. Numerous varieties of soap, lotion, and toothpaste contain *E. globulus* (L.) oil as an antibacterial ingredient. Numerous laboratory studies have shown components in *E. globulus* (L.) oil that have potent antibacterial properties on both fungi and bacteria. Unfortunately, the majority of the wound treatments that are currently available come with their own drawbacks, such as high costs, the emergence of germ resistance, and allergic responses. Finding a safe and efficient alternative to synthetic chemicals from natural resources that are thought to have only few negative side effects is necessary. These encouraged us to investigate the potential activity of pure *E. globulus* (L.) oil and a mixture of *E. globulus* (L.) oil with citronellol, pinene, and linalool on the healing of *Candida albicans* contaminated wounds in rats (El-Sakhawy*et al.,* 2023).

Using plants with medicinal properties to treat wounds have been found useful in fighting against infection and accelerate wound healing (Dogan, 2019) (Fabio et al., 2015). Many medicinal plants have antioxidant and antibacterial properties. *AzadirachtA.indica* (L), a Meliaceae family tree, has been used in India for many years in the treatment of several diseases in medicine and dentistry. Almost all parts of the *A. indica* (L.) Plant are used for medicinal purposes and for the treatment of inflammation, infections, fever, skin diseases, dental problems, and diabetes. *A. indica* (L.) extract contains

DOI 10.17605/OSF.IO/STR76
Vol: 60 | Issue: 05 | 2023
Asma Ahmed et al,. (2023)

flavonoids, alkaloids, steroids, saponins, and tannins. Few studies showed that *A. indica* (L.) oil could be used for chronic non-healing wounds (Jayalakshmi et al., 2021)

Syzygium cumini (L.) has been traditionally used as a medicinal plant. Different parts of the plant (for example bark, leaves, seeds, and fruit) have been employed in the treatment of various diseases. *S.cumini* (L.) fruit juice has been utilized, orally, to treat gastric complaints, diabetes, and dysentery. *S.cumini* (L.) seeds have been applied externally to treat ulcers and sores, and powdered seeds with sugar have been given orally to combat dysentery. Powdered seeds have been reported to be effective against diabetes. *S.cumini* (L.) leaves were cooked in water (concentration of 2.5 g/L) and drunk daily, where 1 L has been reported to be effective against diabetes. The juice of leaves has been used as an antidote in opium poisoning, and an oral intake of leaves for 2–3 days has been reported to be effective in reducing jaundice in adults and children. Traditionally, *S.cumini* (L.) leaves juice along with mango leaves and myrobalan fruit administered with honey and goat milk has been used also to combat dysentery, whereas bark decoction of *S.cumini* (L.) with water has been used to treat diabetes, dysentery, to increase appetite, to achieve sedation, and to relieve headache when taken orally. Bark decoction has been given to females with recurrent miscarriages. *S.cumini* (L.) bark juice with buttermilk has been reported to treat constipation, whereas an intake in the morning has been claimed to stop blood discharge in feces (Qamar *et al.,* 2022).

MATERIALS AND METHODS

Study site

Present experimental analysis was performed at the animal house of Institute of Molecular Biology & Biotechnology (IMBB), The University of Lahore, Lahore, and Punjab, Pakistan. All the materials utilized for the present research work were of analytical grade.

Collection and Identification of plant materials

Fresh leaves of neem, jamun, Eucalyptus and tobacco (Table 1) were collected from the local areas of Lahore, Punjab, Pakistan. Two Herbariums were prepared, out of which one was submitted to the Government College University (GCU), Lahore Punjab Pakistan for the complete identification and voucher numbers while other has been preserved at the host institute for further processing.

Table 1: Details of collected plants

Sr No.	Local names of plants (in Pakistan)	Symbolic Keys
1.	Neem	NP1
2.	Jamun	JP2
3.	Eucalyptus	EP3
4.	Tobacco	TP4

Preparation of experimental plant extracts

After identification, polarity based extracts of all plants were prepared, in which 10.0 g of dried leaves were shade dried at room temperature, followed by grinding of dried leaves into powdered form (80 mesh) by mechanical means. Than n-hexane (Sigma Aldrich, analytical grade) was added in 1:10 ratio and kept for shaking for 24 hours in shaker incubator (K-J-201BD), followed by the centrifugation for 15 minutes at 5000 rpm (SIGMA 203,43191) and filtration through Whatmann filter paper 1.0. Filtrate had been shade dried at room temperature while next solvent (ether, ethanol and dist. water respectively) has been added in residue with repetition of previous procedure. Dried filtrate has been re-dissolved in 15 % DMSO to prepare stock solution (1.0 mg/ ml) (Abu Bakar and Haque, 2020).

DOI 10.17605/OSF.IO/STR76
Vol: 60 | Issue: 05 | 2023
Asma Ahmed et al,. (2023)

Animal selection

After ethical approval (Approval No: USM/Animal Ethics approval/2009), adult male and female albino rats (250-300gm) had been housed in standard stainless steel cages separately to avoid mating, at controlled room temperature and 60-70 % relative humidity and fed with standard laboratory diet with free access to water. Sick and feeble rats were excluded from the study. Animals were divided in following groups;

- **Group 1:** Vehicle (received water only).
- **Group 2:** Negative Control group.
- **Group 3:** Positive control group. Applied with puyodine ointment after the formation of wound

Group 4 to group 19 were experimental groups and were treated with leave extracts of plants

- **Group 4:** Treated with n-hexane extract of *A. indica* (L.)
- **Group 5:** Experimental group. *A.indica* (L.)+ ether extract
- **Group 6:** Experimental group. *A.indica* (L.)+ ethanolic extract
- **Group 7:** Experimental group. *A.indica* (L.)+ water extract
- **Group 8:** Experimental group. *N. tabacum* (L.)+ n-hexane extract
- **Group 9:** Experimental group. *N. tabacum* (L.)+ ether extract
- **Group 10:** Experimental group. *N. tabacum* (L.) + ethanolic extract
- **Group 11:** Experimental group. *N. tabacum* (L.) + water extract
- **Group 12:** Experimental group. *E. globulus* (L.)+ n-hexane extract
- **Group 13:** Experimental group. *E. globulus* (L.)+ ether extract
- **Group 14:** Experimental group. *E. globulus* (L.)+ ethanolic extract
- **Group 15:** Experimental group. *E. globulus* (L.) + water extract
- **Group 16:** Experimental group. *S.cumini* (L.) + n-hexane extract
- **Group 17:** Experimental group. *S.cumini* (L.) + ether extract
- **Group 18:** Experimental group. *S.cumini* (L.) + ethanolic extract
- **Group 19:** Experimental group. *S.cumini* (L.) + water extract

Treatment and Excision on Rats

After induction of anesthesia (40 mg/kg ketamine), a wound (2 × 2 cm) was made by a scalpel, which removed all cutaneous layers of animal. Than rats were randomly divided into above groups and applied the relevant treatments to the wound bed for 14 days. On day 7, the wound contracture (%) was measured by tracing the wound on transparent paper and a permanent marker. Recorded wound areas were measured using 1.0 mm^2 scale of graph paper. Changes in wound area were evaluated after 07, 14 and 21 days, by giving an indication of the rate of wound contraction period. The evaluated surface area was used to calculate the percentage of wound contraction by using following formula, by taking initial size of the wound as 100% **(**Sudheesh *et al.,* 2012**).**

ISSN：1674-2974 | CN 43-1061 / N

DOI 10.17605/OSF.IO/STR76
Vol: 60 | Issue: 05 | 2023
Asma Ahmed et al,. (2023)

Wound contracture (%) = (Wound area on 1st day−Wound area on last day)
Wound area on 1st day × 100.

Statistical analysis

Data has been analyzed through Two-way ANOVA, followed by the calculation of means and standard deviation (SD) through GraphPad 8.0 and results has been expressed as mean± SD.

RESULTS

Wound healing potential of control groups

Identified plants were *Syzygium cumini* (L.) (GC Herb. Bot. 3976), *AzadirachtA.indica* A. Juss (GC Herb. Bot. 3980), *Eucalyptus globulus* Labill (GC Herb. Bot. 3977), *Nicotiana tabacum* (L.) (GC Herb. Bot. 3975). Statistically analyzed results showed that wound healing potential in male rat was more evident as compared to female rat in control groups as animals in the vehicle showed 77.08% wound healing, which may be because of the natural immunity of animals, while male animals in Group III showed 82.56% wound healing and female rats showed 91.45% {Figure 2 (E)}.

Wound Healing Potential of plant extracts

Statistically analyzed results of showed that after day seven, *A.indica* (A.) extracts showed more healing activity in female rats as compared to male rats, especially by ether extract (32%) as compared to n-hexane, water and ethanolic extracts (up-to 25% wound closure only). After day 14, ether and water extracts of *A.indica* (A.) 75% wound closure as compared to other extracts in female rats, followed by water extract with 70% wound contraction in male rats. After day 21, ethanolic extract of *A.indica* (A.) showed best wound contraction (95%), followed by ether extract (92% of wound closure) in female rats, while its n-hexane and water extracts showed 90% healing in females and 82% and 87% respectively in male rats {Figure 1 (A) and Figure 2 (A)}

After one week, ethanolic extract of *E. globulus* (Labill.) showed 70% of wound closure in female rats followed by 57.5% wound healing in male rats. 52.5% and 47.5% of wound healing was seen in male and female rats respectively with ether extract of this plant after one week. After two weeks, ether extract of *E. globulus* (Labill.) showed 95% wound closure in female rats followed by ether and n-hexane extracts with 92.5% of wound closure in female rats. 90% of wound closure was shown by water extract in female rats followed by 72.5% of wound healing in male rats by n-hexane extract. After three weeks, 100% wound closure has been observed by all extracts of *E. globulus* (Labill.) leaves in female rats, while only n-Hexane extract have shown 95% of wound healing in male rats {Figure 1 (B) and Figure 2 (B)}.

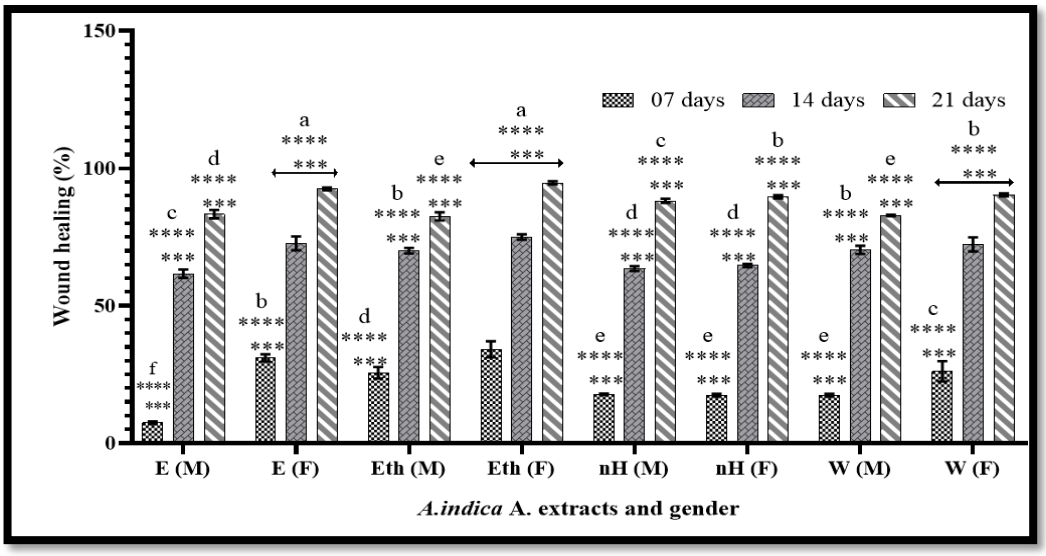
After day 7, 40% of wound closure was observed with ether and water extracts of *N. tabacum* (L.) in male rats, followed by 35% of wound healing in female rats. Ethanolic and n-hexane extracts of this plant showed least healing in both male and female rats. After day 14, statistically analyzed results of water extract of *N. tabacum* (L.) showed promising results in both male and female rats with 87.5% and 86% wound closure respectively while ether extract showed 85% wound healing followed by ethanolic extract with 75% wound contraction in male rats as compared to female rats (70%). After day 21, water extract of showed such promising results in male rats as compared to female rats (95% of wound closure in male rats and 91% of wound closure in female rats). Its ethanolic and n-hexane extracts also showed 90% of wound healing in both male and female rats. Least wound healing potential has been shown by ether extract with 82.5% in male rats {Figure 1 (C) and Figure 2 (C)}.

After seven days, ether extract of *S.cumini* (L.) showed 55% wound healing, followed by its n-hexane extract which showed 50% wound healing in female rats. 37.5% wound healing was shown in male

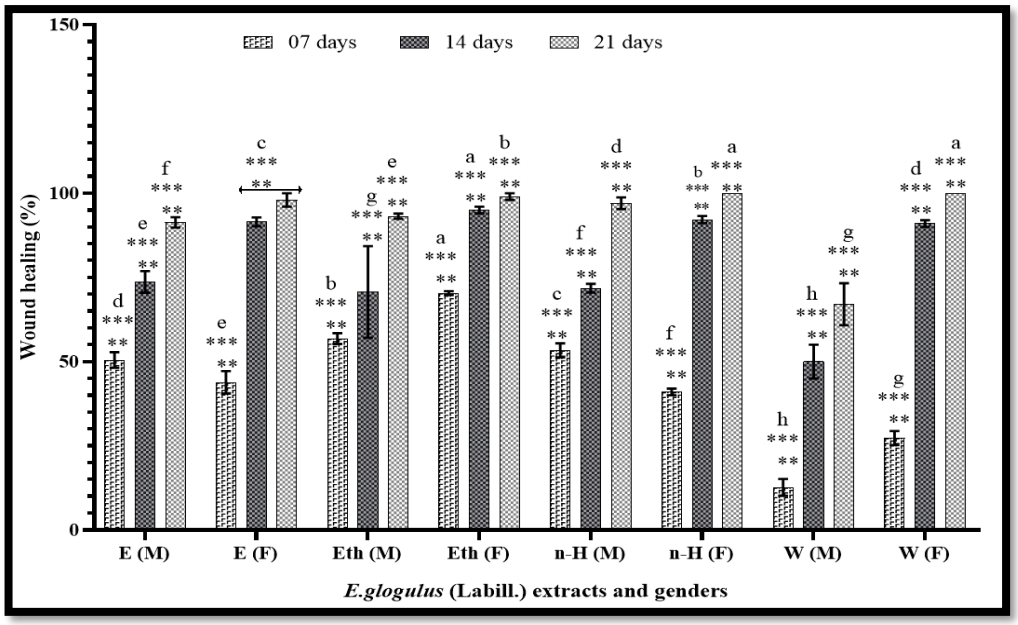
ISSN：1674-2974 | CN 43-1061 / N

DOI 10.17605/OSF.IO/STR76
Vol: 60 | Issue: 05 | 2023
Asma Ahmed et al,. (2023)

rats with water extract followed by the ethanolic extract (35% wound healing). After fourteen days, n-Hexane extract of *S.cumini* (L.) showed 92.5% wound healing in female rats followed by its ether and water extracts (90% wound closure in female and male rats respectively). 87.5% and 80% of wound closure has been observed in female and male rats respectively when they had been treated with water and n-hexane extracts respectively. After 21 days, all four extracts of *S.cumini* (L.) showed promising results with 100% wound closure in both male and female rats, followed by its ether and ethanolic extracts (90% and 85% wound closure respectively) in male rats {Figure 1 (D) and Figure 2 (D)}.



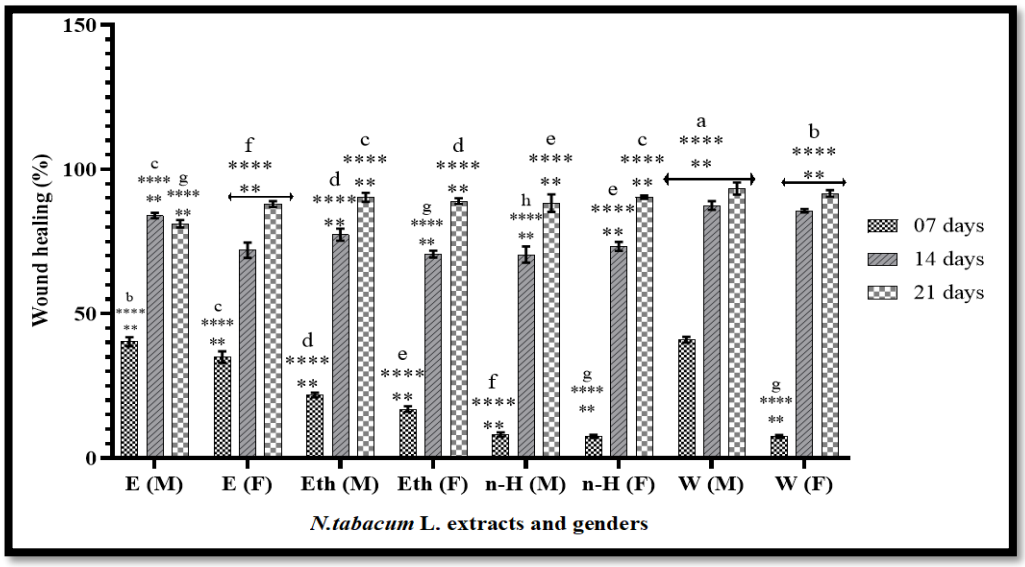
(A)



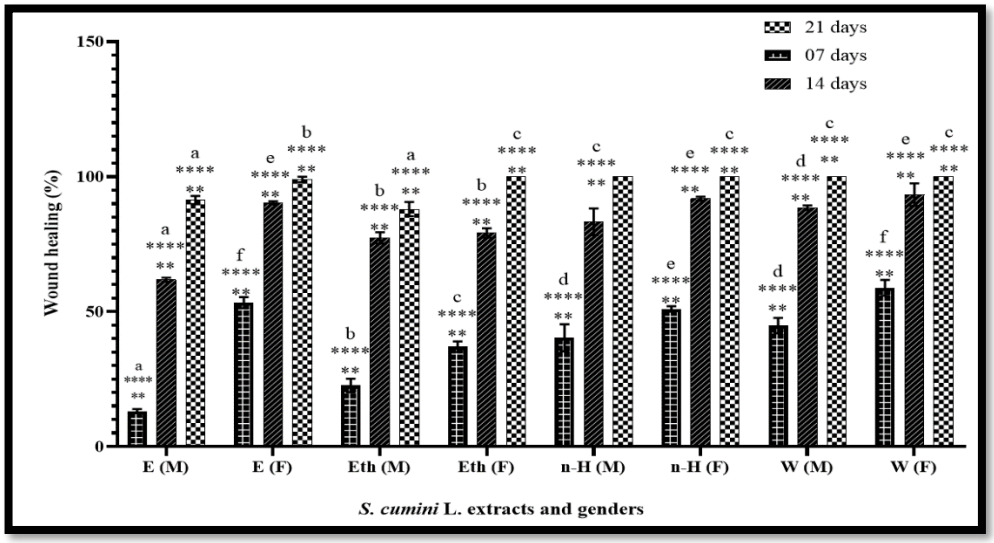
(B)

ISSN：1674-2974 | CN 43-1061 / N

DOI 10.17605/OSF.IO/STR76
Vol: 60 | Issue: 05 | 2023
Asma Ahmed et al,. (2023)



(C)



(D)

Figure 1: Wound healing effect of leaf extracts of plants on different genders of albino Wistar rats

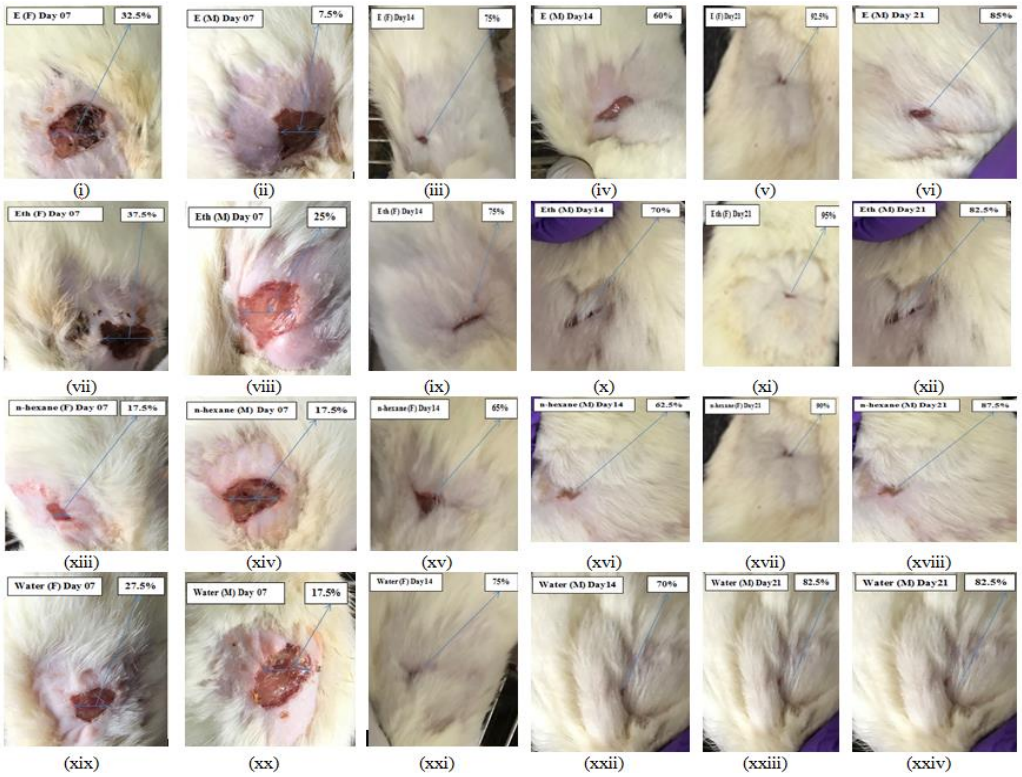
(A) *A. indica* A. Extracts (B) *E. globulus* Labill. Extracts (C) *N.tabacum* (L.) Extracts (D) *S.cumini* L. Extracts

M= Male F= Female E= Ether extract, Eth= Ethanolic extract, n-H= n-Hexane extract, W= Water extract, **a** to **h** shows maximum to minimum wound healing efficacy (%)

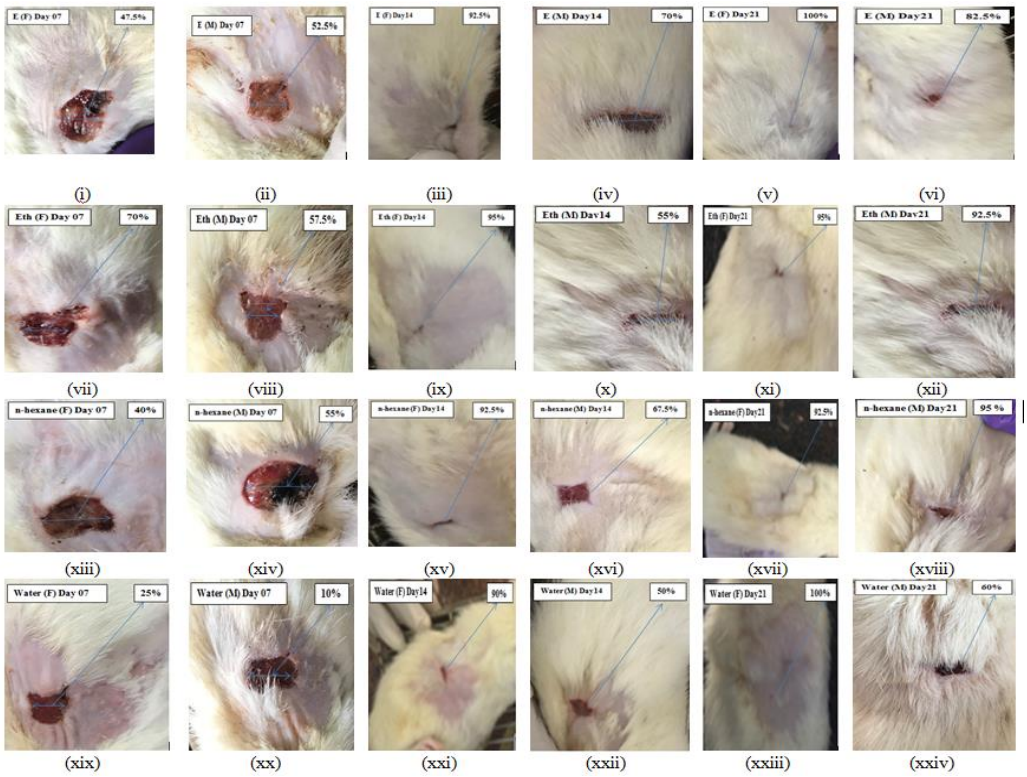
** = Significant wound healing effect (%) on different days by leaf extracts of *N.tabacum* (L.) and *S.cumini* (L.) at p= 0.0017 and *E.globulus* (L.) at p= 0.0044, ***= Highly significant wound healing effect (%) by leaf extracts of *A. indica* (L.) among different days (p= 0.0008) and among different extracts of *E.globulus* (L.) (p= 0.0009), **** = Extremely significant wound healing effects (%) among different extracts of *A. indica* (L.), *N.tabacum* (L.) and *S.cumini* (L.) at p< 0.0001.

ISSN：1674-2974 | CN 43-1061 / N

DOI 10.17605/OSF.IO/STR76
Vol: 60 | Issue: 05 | 2023
Asma Ahmed et al,. (2023)



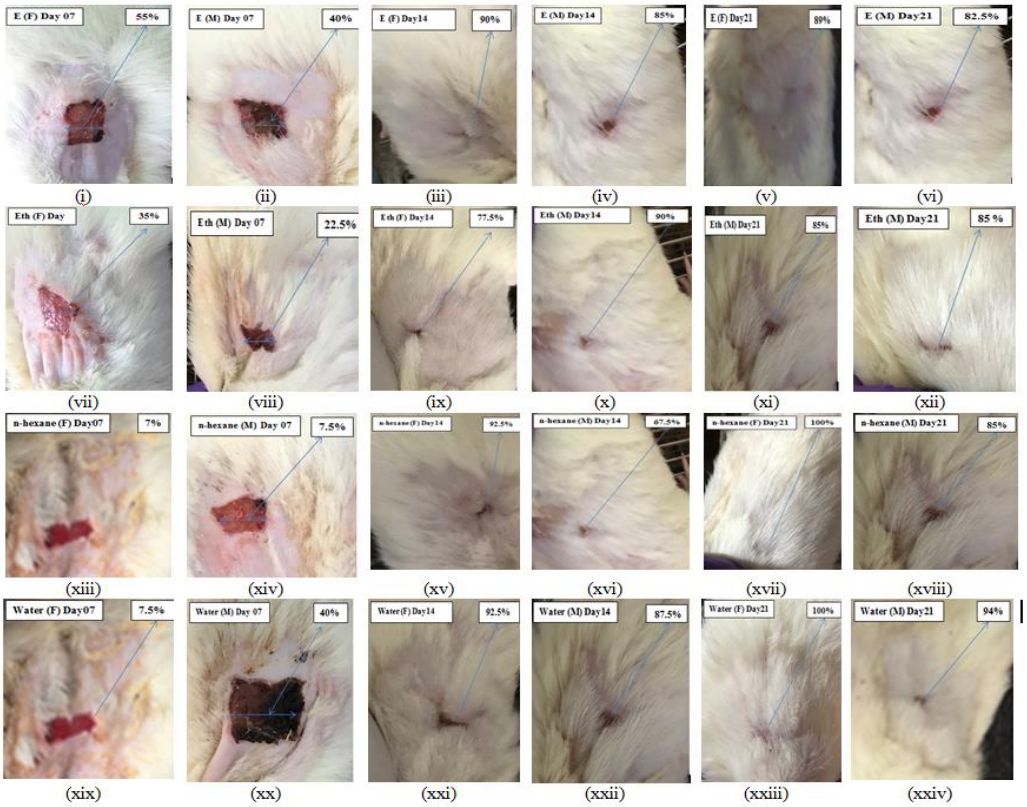
(A)



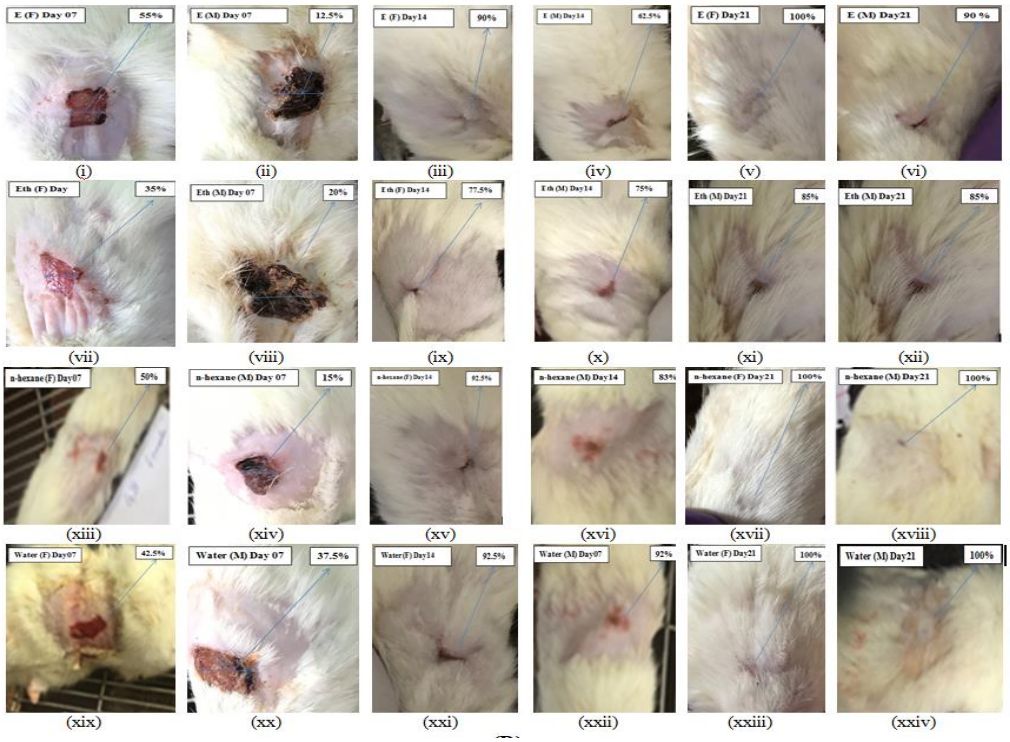
(B)

ISSN：1674-2974 | CN 43-1061 / N

DOI 10.17605/OSF.IO/STR76
Vol: 60 | Issue: 05 | 2023
Asma Ahmed et al,. (2023)



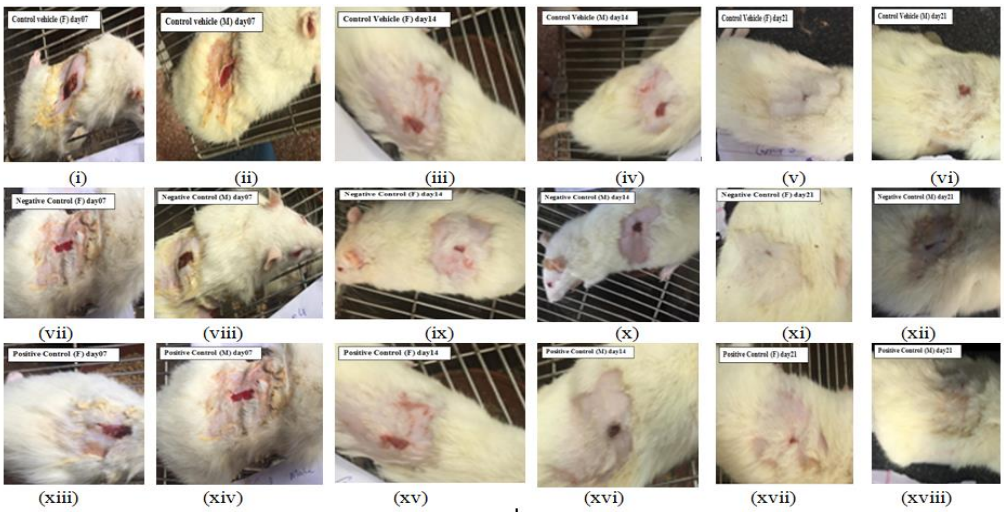
(C)



(D)

ISSN：1674-2974 | CN 43-1061 / N

DOI 10.17605/OSF.IO/STR76
Vol: 60 | Issue: 05 | 2023
Asma Ahmed et al,. (2023)



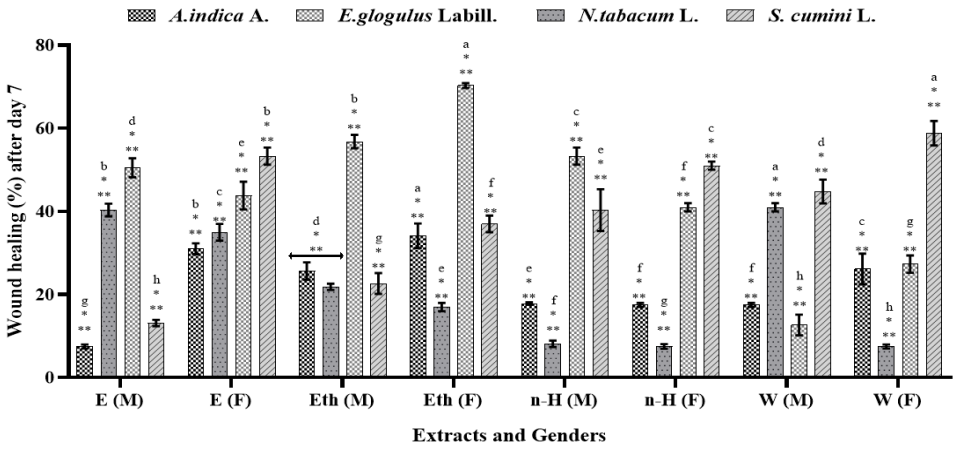
(E)

Figure 2: Wound healing effect (%) of leaf extracts of plants on different genders of albino Wistar rats

M= Male F= Female E= Ether extract, Eth= Ethanolic extract, n-H= n-Hexane extract, W= Water extract, %= wound healing efficacy
(A) *A. indica* (A.) (B) *E.globulus* (Labill.) (C) *N.tabacum* (L.) (D) *S.cumini* (L.) (E) Control groups

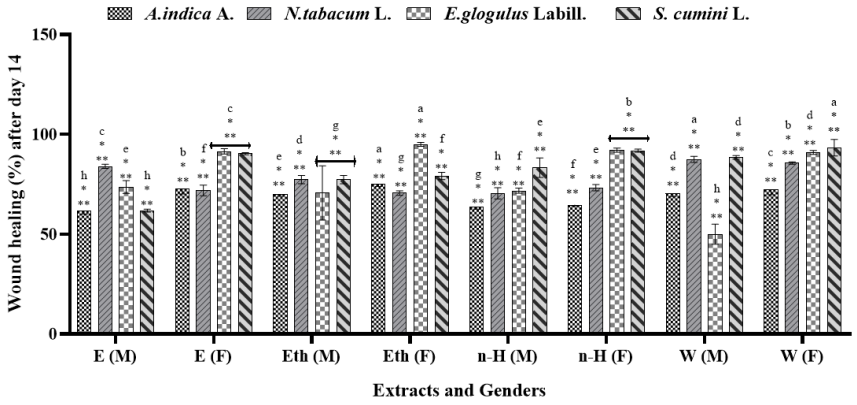
Comparison of wound healing potential of all plant extracts

After statistical analysis, it had been observed all extracts of *A.indica* (A.) leaves showed significant wound healing activity on different intervals of days, out of which ethanolic extract has most profound wound healing especially in female rats as compared to male rats. Among all extracts of *S.cumini* (L.) leaves, water extracts have significant wound closure in female rats as compared to male rats. Statistically observed results proved that all extracts of *E. glogulus* (Labill.) leaves had highest wound healing activity especially by ethanolic extract in female rats and least wound healing activity were observed in male rats by the water extract of this plant. Statistical analysis of all extracts of *N. tabacum* (L.) showed that highly significant wound healing activity (with respect to days) has been shown in both male and female rats with water extract, while n-Hexane extract had shown least significant results in both male and female rats (Figure 3).

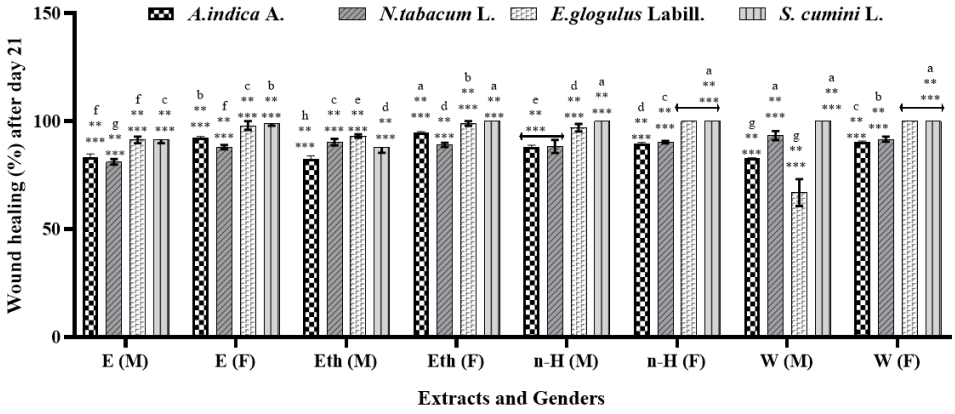


(A)

DOI 10.17605/OSF.IO/STR76
Vol: 60 | Issue: 05 | 2023
Asma Ahmed et al,. (2023)



(B)



(C)

Figure 3: Comparison of wound healing effect (%) of leaf extracts of plants on different genders of albino Wistar rats

(A)= After day 7, (B) = after day 14, (C) = after day 21

M= Male F= Female E= Ether extract, Eth= Ethanolic extract, n-H= n-Hexane extract, W= Water extract, **a** to **h** shows maximum to minimum wound healing efficacy (%).

* = Significant wound healing effect (%) among *N.tabacum* (L.), *S.cumini* (L.), *A. indica* (L.) and *E.globulus* (L.) at p= 0.0110 after day 07 and p= 0.0155 after day 14, **= Moderately significant wound healing effect (%) among *N.tabacum* (L.), *S.cumini* (L.), *A. indica* (L.) and *E.globulus* (L.) after day 21 (p= 0.0042) and among different extracts of all plants after day 07 (p= 0.0011) and 14 (p= 0.0027), *** = Highly significant wound healing effects (%) due to different extracts of all plants after day 21 at p= 0.0006.

DISCUSSION

Wound healing is dynamic process and complicated that restores cellular structures and tissue layers in damaged tissue to their normal state at its best. Wound contracture is a condition that happens throughout the healing process, beginning with the fibroblastic stage, in which the wound shrinks. Hemostasis and inflammation characterize the inflammatory phase, which is followed by epithelialization, angiogenesis, and collagen deposition in the proliferative phase. The wound

DOI 10.17605/OSF.IO/STR76
Vol: 60 | Issue: 05 | 2023
Asma Ahmed et al,. (2023)

contracts during the maturational phase, the final stage of wound healing, results in lower quantity of apparent scar tissue (Nilesh *et al.,* 2010).

Somwanshi, S.B., and Hiremath, S. N. (2018) found that the utilization of herbal plants and less toxic nature implies that they can be managed over extensive stretches. Significance of poly herbal medications are better contrasted with synthetic chemicals alone on the grounds that polyherbals contain different poly constituents that have calming, cell reinforcement, antimicrobial properties and show synergistic impact on process of wound healing. Consequently phytochemicals show better wellbeing, adequacy and lack of unfavorable response. Hence logical scientific examination is extremely necessary to investigate the pharmacological use herbal meds and to clarify the cases made with regards to them in traditional prescriptions.

Studies have confirmed that collagen accumulation, granulation, epithelialization and wound contraction are all characteristics of wound heading's proliferative phase. Myofibroblasts help in contraction, and epithelial cells migration across the wound site to heal it. Throughout the healing process, wound contraction occurs. When *A. indica,* A., and *N. tabacum* L., extracts were used in experimental groups, the rate of wound contraction increased as compared to the control group and rapid epithelialization and collagenization has been observed at the 14^{th} day of wound creation, which showed that collagen synthesis has improved, and thus indicating the effective healing. Epithelialization occurs after an injury when epithelial cell proliferate and shift towards the wound site while wound contraction is mostly because of the action of myofibroblasts (Shivananda *et al.,* 2014).

Collagen is a major component of connective tissues, which creates the basic scaffolding for regenerative tissues and it plays a vital role in wound healing. Collagen is primarily made up of amino acid hydroxyproline, which is a prominent component of the extracellular matrix, gives the tissue strength and support. Methanolic extract significantly improved wound contraction in the experimental group as compared to the control group. As a result, it can be anticipated that methanolic extract boosts the rate of collagen synthesis, resulting in faster wound healing, which is attributed to the phytocompounds in Methanolic extracts of plant (Table 2) (Samanta*et al.,* 2016). Phyto-extracts of *A. indica,* A., *N. tabacum* L., *E. globulus* L., and *S. cumini* L. leaves in different solvents ether, ethanol, n-hexane and water produced significant wound healing activity and multiple studies have reported that phytochemicals from *A. indica*, A., *N. tabacum* L., *E. globulus,* L., and *S. cumini,* L. can promote wound healing through their anti-inflammatory, antioxidant, antimicrobial, and immunomodulatory properties. *A. indica*, A. is rich in triterpenoids, flavonoids, and phenolic compounds, which have been shown to have anti-inflammatory, antimicrobial, and antioxidant properties, and all these features are compulsory for wound healing. *N. tabacum* L., contain nicotine, alkaloids, and coumarins, which are known to have anti-inflammatory, antimicrobial, and antioxidant properties. *E. globulus*, L., contains cineole, camphor, and other volatile oils, which are also antimicrobial, anti-inflammatory, and antioxidant in nature. *S. cumini,* L., have tannins, flavonoids, and phenolic compounds, which have been reported to have anti-inflammatory and antioxidant properties (Table 2). These phytochemicals are effective in promoting wound healing by increasing the rate of healing, reducing inflammation, and providing protection against infection (Kumar &Ahirwar 2017)

DOI 10.17605/OSF.IO/STR76
Vol: 60 | Issue: 05 | 2023
Asma Ahmed et al,. (2023)

Table 2: Phyto-compounds and their properties in polarity based extracts of *A. indica, L., N. tabacum* L., *E. globulus*L., and *S. cumini* L. leaf extract

Compounds	Molecular Formulae	Molecular Weight g/mol	Structures (Figure 4)	Solubility	Pub Chem Id
Saponins	$C_{58}H_{94}O_{27}$	1223.3	a	Polar/soluble	198016
Quinones	$C_{15}H_{14}O_6$	290.27	b	Less polar/less soluble	11437738
Oxalic Acid	$C_2H_2O_4$	90.03	c	Polar/ soluble	12252960
Gallic Acid	$C_7H_6O_5$	170.12	d	Polar/ soluble	288114
Taxifolin	$C_{15}H_{12}O_7$	304.25	e	Polar/soluble	439533
Methyl Gallate	$C_8H_8O_5$	184.147	f	Polar/soluble	7428
Quercetin	$C_{15}H_{10}O_7$	302.236	g	Polar/ soluble	5280343
Luteolin	$C_{15}H_{10}O_6$	286.24	h	Polar/ soluble	5280445
Hesperidin	$C_{28}H_{34}O_{15}$	610.565	i	Less polar/ less soluble	10621

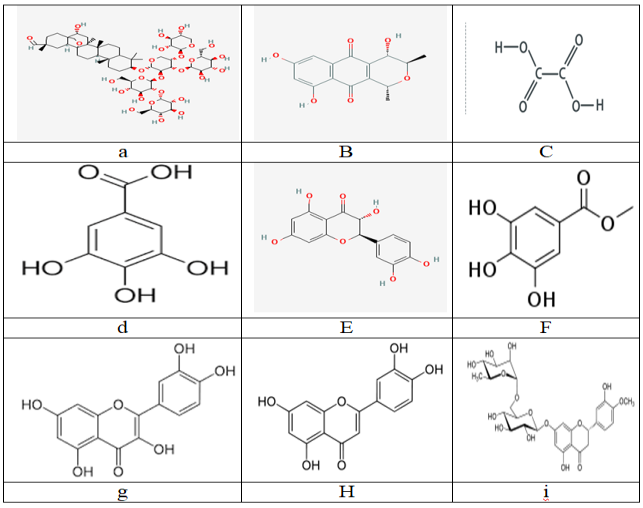


Figure 3: Major Phytocompounds in Different Extracts of Experimental Plants

CONCLUSION

It had been concluded that ethanolic and water extracts of *A.indica* (A.), *E. glogulus* (Labill.) and *S.cumini* (L.) and *N. tabacum* (L.) leaves respectively showed significant wound healing activity on different intervals of days in female rats as compared to male rats, while n-Hexane extract of all plants have least significant effect on wound contraction. This shows that this wound contraction is attributed to polar compounds which are present in ethanolic and water extracts of these plants.

Competing Intersts

N/A

Acknowledgement

All authors are highly acknowledged to Government of Pakistan which have approved the the host institution and Higher Education Commission (HEC) Pakistan, under the umbrella of which HEC approved Ph.D. faculty has executed this project as a part of Ph.D. thesis.

Funding Source

N/A

DOI 10.17605/OSF.IO/STR76
Vol: 60 | Issue: 05 | 2023
Asma Ahmed et al,. (2023)

References

1) Abubakar AR, Haque M. (2020). Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. Journal of Pharmacy & Bioallied Sciences; 12 (1): 1.

2) Banozic, M, Banjari I, Jakovljević M, Subaric D, Tomas S, Babic, J, &Jokic, S. (2019). Optimization of ultrasound-assisted extraction of some bioactive compounds from *N. tabacum* (L.) waste. *Molecules*; *24*(8): 1611.

3) Banozic, M, Subaric, D, Jokic S, (2018). *N. tabacum* (L.) waste in Bosnia and Herzegovina–problem or high-value material? Glasnikzastitebilja 4; 24–33.

4) Cvetanovska A, Krstic M, Cvetanovska L, (2017). Content of total phenolic compounds and antioxidant potential of oriental *N. tabacum* (L.) varieties (*Nicotiana tabacum* (L.). European Journal of Pharmaceutical and Medical Research; 4, 2223–2228.

5) Dogan KH (Ed.) (2019). Wound Healing: Current Perspectives.

6) El-Sakhawy MA, Soliman GA, El-Sheikh HH, & Ganaie MA (2023). Anticandidal effect of *Eucalyptus globulus* oil and three isolated compounds on cutaneous wound healing in rats. European Review for Medical and Pharmacological Sciences; *27*, 26-37.

7) Fabio G, Romanucci V, Marino C, Pisanti A, and Zarrelli A & Gymnemasylvestre R Br, (2015). An Indian medicinal herb: traditional uses, chemical composition, and biological activity. Current Pharmaceutical Biotechnology; 16(6) 506–516 2015.

8) Jayalakshmi MS, Thenmozhi P, &Vijayaraghavan R. (2021). Plant leaves extract irrigation on wound healing in diabetic foot ulcers. *Evidence-Based Complementary and Alternative Medicine*; 1-9.

9) Mohandas A, Sudheesh Kumar PT, Raja B, Lakshmanan, VK, & Jayakumar R. (2015). Exploration of alginate hydrogel/nano zinc oxide composite bandages for infected wounds. International journal of nanomedicine; 10(Suppl 1), 53.

10) Nilesh S, Amit J, Vaishali U, Sujit K, Sachin K, & Ravindra P. (2010). Protective effect of *Lepidium sativum* against doxorubicin-induced nephrotoxicity in rats. *Research Journal of Pharmaceutical Biological and Chemical Sciences*; *1*(3), 42-49.

11) Qamar M, Akhtar S, Ismail T, Wahid M, Abbas MW, Mubarak MS, & Esatbeyoglu T (2022). Phytochemical Profile, Biological Properties, and Food Applications of the Medicinal Plant *Syzygium cumini*. *Foods*; *11*(3), 378.

12) Ravindran J, Arumugasamy V, & Baskaran A. (2019). Wound healing effect of silver nanoparticles from *Tridax procumbens* leaf extracts on Pangasius hypophthalmus. *Wound Medicine*; *27*(1), 100170.

13) Samanta R, Pattnaik AK, Pradhan KK, Mehta BK, Pattanayak SP, and Banerjee S. (2016). Wound healing activity of silibinin in mice. *Pharmacognosy research*; *8*(4), 298.

14) Sharma A, Khanna S, Kaur G, & Singh I. (2021). Medicinal plants and their components for wound healing applications. *Future Journal of Pharmaceutical Sciences*; *7*(1), 1-13.

15) Somwanshi SB, and Hiremath, SN. (2018). In-vivo Evaluation of the Wound Healing Activity of the *Sesamum Indicum* L. Seed Extract in Novel Ethosomal Vesicular System. *Journal of Drug Delivery and Therapeutics*; *8*(5), 411-420.

16) Sourirajan A, Dev K, & Kumar V. (2020). Comparison of extraction yield, phytochemicals and in vitro antioxidant potential of different extracts of two Ficus species. *Plant Archives*; *20*(1), 2669-2673.

17) Sudheesh Kumar PT, Lakshmanan VK, Anilkumar TV, Ramya C, Reshmi P, Unnikrishnan AG, & Jayakumar R. (2012). Flexible and microporous chitosan hydrogel/nano ZnO composite bandages for wound dressing: *in vitro* and *in vivo* evaluation. *ACS applied materials & interfaces*; *4*(5), 2618-2629.