Original Article

Extraction, Isolation Of Chemical Compounds And Evaluation Of Wound Healing Activity Of *Bombax Ceiba* Plant

Neetu[1*], S. Ramasamy[2], Vinod Kumar Singh[3]

[1*]Research Scholar, Department of Pharmacy, MJP Rohilkhand University, Bareilly IN
[2]Associate Professor, Department of Pharmacy, MJP Rohilkhand University, Bareilly IN
[3]Research Scholar, Department of Pharmacy, Integral University, Lucknow IN

***Corresponding Author:** Neetu

*Research Scholar, Department of Pharmacy, MJP Rohilkhand University, Bareilly IN, Email id: neetupatel2131997@gmail.com
DOI: 10.47750/pnr.2022.13.S08.653

Abstract

Herbal plants have a lot of benefits over conventional drugs, including the fact that they usually have less frequent side-effects and might be non-toxic if used for a long time. *B. Ceiba* is an enormous, stunning, and deciduous tree and native for Northern Australian origin. The present study was based on the extraction, isolation of chemical compounds and evaluation of wound healing activity of *Bombax ceiba* plant. The chemicals and instruments were obtained from the certified manufacturer and suppliers only. *B. ceiba* roots and flowers were collected and authenticated from a Botanist at MJPRU Bareilly. The extraction process was done by maceration process. Pharmacological (wound healing) potential was evaluated by wound contraction, re-epithelization, and histopathology. The n-hexane, chloroform, ethyl acetoacetate and methanol extracts of B. ceiba were examined for its phytoconstituents. Wistar rats weighing 130-210g were obtained from the Dept. of Pharmacy, MJP Rohilkhand University Bareilly. The % yield was obtained as 59% for n-hexane, 62% for chloroform, 58% for ethyl acetoacetate and 65% for methanol. According to the preliminary phytochemical analysis, the methanolic *B. ceiba* extracts showed excellent phytoconstituents of alkaloids, flavonoids, glycosides, carbohydrates, etc. when compared with other solvents extracts. In results, all the groups of animals showed an excellent wound healing activity when compared with control group after 15th day of exposure with treatments. The *B. ceiba* N-hexane, chloroform, ethyl acetoacetate, and methanol demonstrated rats showed the re-epithelization period of 11.20±0.62**, 12.17±1.30***, 9.3±1.23** and 10.4±1.12***, respectively. It is concluded that each of these has considerably exhibited wound healing potential. It suggests, to isolate the active constituent responsible for the activity.

Keywords: *Bombax ceiba*, wound healing, re-epithelization period, wound contraction, Wistar rats

INTRODUCTION

Herbal plants have a lot of benefits over conventional drugs, including the fact that they usually have less frequent side-effects and might be non-toxic if used for a long time. They are inexpensive and more readily available [1]. Plants have been found to have a lot of potential for treating and managing diseases. Tribal and folklore physicians in lots of countries have employed a variety of plants to treat a variety of diseases [2].

B. Ceiba is an enormous, stunning, and deciduous tree and native for Northern Australian origin. It is extensively dispersed in Africa, Australia, and Asia, both temperate and tropical. Plants develop in the sweltering regions of India, climbing the hills up to 1,500m. The plant can reach a height of 25 to 30 metres and has broad, spreading branches and juvenile stems that are covered in thick, stiff prickles. The bark is 1.8–2.5 cm thick and varies in colour from silver grey to ash [3].

Wound-healing refers an important physiological alteration that requires the collaboration of many different cell types and their products. Attempts to repair the damage produced by local aggression begin early in the inflammatory stage [4]. According to Mitchel et al., healing process exhibits the principles of tissue restoration for many tissues [5]. The stages of cell & biochemical processes in wound repair include inflammatory reaction, cell proliferation & creation of extracellular matrix materials & post-healing period, known as remodelling [6].

Coagulation is the collection of thrombocytes and platelets in a fibrin matrix as a result of certain stimuli activating and accumulating these cells [45]. The lesion destroys tissue by causing inflammation, which is a temporary and defensive tissue response [7]. Angiogenesis refers a co-ordinated process i.e., endothelial-cellular growth, basal layer damage etc. [8]. About 4 days post-lesion, rolled tissue starts to form network. It gets its name because of the granular presence in latest developed tissue [9]. Re-modelling refers 3rd phase in healing which starts 2-3 weeks post initiation of the lesion (stay ≥1 year) [10].

MATERIALS AND METHODS

Experimental requirements

Fresh dried roots & flowers of *B. ceiba*, methanol, n-hexane, ethyl acetate, chloroform, distilled water, rotatory evaporator, pestle mortar, desiccator, percolator, petri dishes and Wistar rats.

Collection and authentication of plants

The plant (fresh roots & flowers) was harvested from the Himalaya region. The plant was developed for herbarium and authenticated by the Botanist at Plant Science Dept. MJP Rohilkhand University Bareilly. The roots & flowers were shade dried, crushed, and weighed for equal amounts of 10g. They both were mixed and soaked into 500ml of n-hexane, chloroform, ethyl acetoacetate and methanol solvents (differently) for 1 week. After a week, all the macerates were filtered off to get the extracts of n-hexane, chloroform, ethyl acetoacetate and methanol, respectively.

Phytochemical study

For Alkaloids

Dragendroff's test: Plant extract is dissolved in chloroform. Add a few drops of Dragendroff's agent to the chloroform residual after it has been acidified and evaporated. Alkaloids are present if orange red precipitate is present.
Mayer's test: When Mayer's reagent is added to 2-3 ml of filtrate, it gives ppt.
Wagner's test: Wagner's reagent is added to 2-3 ml of filtrate, it gives a reddish-brown appearance.
Hager's test: When a few drops of the Hager's reagent were added to plant extracts, a precipitate with a yellow hue formed, indicating the presence of alkaloids.

For Saponins

Foam test: Place a tiny amount of extract and water in a test tube. Shake ferociously the presence of Saponin is indicated by the formation of foam that lasts for 10 minutes.
Test for carbohydrates

Fehling's test: One milliliter of each mixture of Fehling's A and B solutions boils for a minute. To the test extract solution, add an equal amount. 5–10 minutes in a water bath. An orange-red colour precipitate appears that suggests the presence of carbohydrates.

Benedict's test: In a test tube, add test extract in an equal volume to Benedict's reagent. Heat for five minutes in a boiling water bath. The resolution seems green. Depending on how much disaccharide is in the test solution, the colour will either be yellow or red.

Test for flavonoids

Ferric chloride test: Add a few drops of neutralized fecl3 to the extract's alcoholic solution. The presence of flavonoids is indicated by the color green.

Shinoda test: To a dry extract, add 5ml of 95% ethanol, a few drops of hydrochloric acid, and 0.5g of magnesium powder. The color pink is visible.
Lead acetate solution test: Add a few drops of lead acetate to the test solution, and it gives a lemon-green precipitate.

Test for proteins

Biuret test:

Add 2 ml of the biuret reagent to the extract, mix it thoroughly, and warm it over a water bath. Proteins are present if they have a red or violet color. Add a few drops of 15 CuSO4 solution and 4 percent NaOH to 3 mL of extract. It appears pink or violated.

Million's test: A standard solution mixed with Million's Reagent and then boiled in a water bath produces a reddish-brown color precipitate.

Xanthoprotein test: The test solution is subjected to intense nitric acid treatment and boiling, producing a yellow precipitate.

Ninhydrin test: A blue hue is produced when the test solution is exposed to the Ninhydrin reagent.

Test for Glycosides

Keller-Kelliani test: The test solution was then added to 2 ml of ferric chloride solution along with a few drops of glacial acetic acid. Sulphuric acid is dripped into the test tube's sidewalls, causing the upper layer to become blue-green and the two layers to split and reveal a reddish-brown color.
Bromine water test: A test solution is dissolved in bromine water; a yellow precipitate appears.
Legal's test: When the test solution is treated with pyridine, it becomes pink to red in color.

Test for amino acids

Test for Tyrosine: warm 3 ml of extract and 3 drops of Million's reagent. A dark crimson color appears in the solution.
Test for cysteine: Add a few drops of a 10% lead acetate and 40% sodium hydroxide solution to 5 ml of the extract. Lead sufate forms as a dark ppt.
Test for steroids
Liebermann's reaction: Add 3 ml of extract to 3 ml of acetic anhydride. Warm and mild. Few drops of concentrated H2SO4 It look to be blue.

Nano-emulsions cream

Nano-emulsions were prepared using the self-emulsification method. Tween 80 and Span 80 as well as ethanol as a co-surfactant, were used. The oil phase consisted of olive oil, whereas the exterior phase consisted of water.
Test for stability
Accelerated storage testing, which included centrifugation, heating, cooling, and a freeze-thaw cycle test, was used to examine the physical stability of *B. ceiba* nano emulsions. The transparency and phase separation of all the formulations were examined visually.

Preparation of nano emulsion cream

An improved nano emulsions containing 10% (w/w) respective extracts were used to make topical herbal cream by completely mixing with the cream base (1:1), yielding the final samples containing 10% (w/w) extract of *B. ceiba*. Liquid & soft paraffin, cetyl alcohol, cholesterol and beeswax were used in preparation of cream base.

Experimental animals

Wistar rats weighing 130-210g were used in this study to assess wound healing potential of *B. ceiba* extract. The rats were obtained from the Dept. of Pharmacy, MJP Rohilkhand University Bareilly. Rats were kept for two-weeks to accommodate in the environment. They were housed in plastic cages (at a constant temperature & RH) at which they were fed a conventional pellet diet *ad libitum*.

Group design

For the purposes of this investigation, the rats were separated into six groups, each with 5 individuals.
Group 1 was applied with cream base.
Group 2 was applied with framycetin ointment.
Group 3 was applied with *B. ceiba* n-hexane extract.
Group 4 was applied with *B. ceiba* chloroform extract.
Group 5 was applied with *B. ceiba* ethyl acetoacetate extract.
Group 6 was applied with of *B. ceiba* methanol extract.
All the treatments were topically applied twice a day for 15 days.

WOUND HEALING PARAMETERS

Wound contraction measurement

On 5^{th}, 10^{th}, 15^{th} day for all groups in the excision wound model, wound area was assessed by tracing the wound with a clear sheet using millimetre-based graph paper. Wound contraction was measured every 5^{th} day until the wound healed completely, and the percentage of healing wound area was calculated with a 100% initial wound size:

$$\text{wound contraction (\%)} = \frac{\text{wound size (prior)- wound size (post)}}{\text{wound size (prior)}} \times 100$$

Re-epithelization

The level of wound healing is assessed using the tensile strength of a healed skin wound. It provides information on the quality of the healing tissue as well as how resilient the restored tissue is to breaking under stress. The sutures were taken out, the tissues that had been healed were excised, and all the animals were given general anaesthesia on the ninth day. On either side of the incision line, a line 3 mm out from the wound was drawn. Two Allis forceps were securely set on the line with their faces toward each other. One forceps was fixed, while the other was connected by a thread to a pulley on a lightweight polypropylene graded container that was hanging freely. Standard weights were softly and progressively added to the container. The borders of the wound were forced apart as the weight was gradually distributed to the incision site. As soon as the cut started to bleed, the weight was stopped and recorded. The number of days it took for dead tissue remnants of the incision to fall off without leaving any open wounds was used to assess the epithelialization time [11].

Histopathology

The tissues from the wound were examined under a compound microscope. In order to identify the pathological abnormalities in the cells seen, tissues were separated and maintained on microscope slides.

RESULTS AND DISCUSSION

Extraction yields

The % yield was obtained as 59% for n-hexane, 62% for chloroform, 58% for ethyl acetoacetate and 65% for methanol calculated with the theoretical and practical. It exhibited that percentage yield was better obtained in methanol.

Phytochemical investigation

According to the preliminary phytochemical analysis, the methanolic *B. ceiba* extracts showed excellent phytoconstituents of alkaloids, flavonoids, glycosides, carbohydrates, etc. when compared with other solvents extracts.

The table 5.2 demonstrates that B. ceiba root and flower extract have numerous phytoconstituents and maximum were seen in ethyl acetoacetate extract when compared with other solvents including n-hexane, chloroform, and methanol. It indicates that ethyl acetoacetate has an excellent solubility of phytoconstituents that might be better lipophilicity.

Saponins were observed through foam test that found in all solvents except the extracted through chloroform. It was also confirmed for alkaloids by employing methods i.e., Mayer's test, Hager's test etc.

Fehling's test was found positive in all the extracts examined that confirmed for the presence of carbohydrates.
Biuret test was found positive in all the extracts except methanol extract that indicates for the presence of protein. Shinoda test was also seen active in n-hexane, chloroform, ethyl acetoacetate and methanol that confirmed for its tannins content.

Keller-killiani test, Bromine water test and Legal's tests were demonstrated as positive that indicated for the presence of glycosides. Cysteine was observed as negative but Test for tyrosine and Ninhydrine test were found in methanol extract. Liebermann's reaction was positive in chloroform, ethyl acetoacetate and methanol extracts besides n-hexane that assured for its presence of Triterpenoids /Steroids.
Results are shown in following table-

Table 1. Phytochemical screening test of *B. ceiba* extract

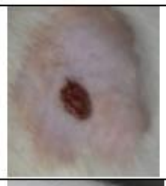
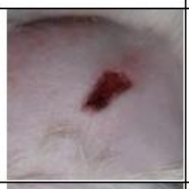
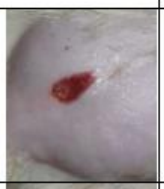
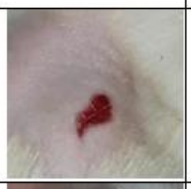
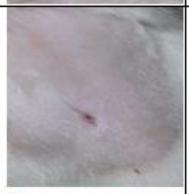
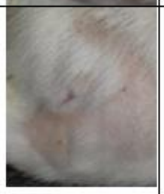
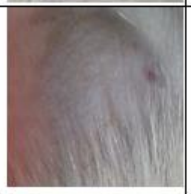
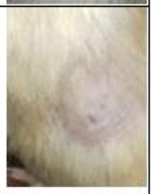
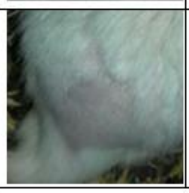
Name of phytochemical test	**Types of tests**	**Inference**			
		n-hexane	chloroform	ethyl acetoacetate	methanol
Saponins					
	Foam test	+	-	+	+
Alkaloids					
	Dragendroff's test	+	+	+	+
	Mayer's test	+	+	+	+
	Wagner's test	+	–	+	–
	Hager's test	–	+	+	–
Carbohydrates					
	Fehling's test	+	+	+	+
	Benedict's test	–	–	–	–
Tannins					
	Ferric chloride test	+	+	+	–
	Shinoda test	+	+	+	+
	Alkaline reagent test	–	–	+	+
	Lead acetate solution test	–	+	–	+
Proteins					
	Biuret test	+	+	+	–
	Million's test	–	+	+	–
	Xanthoprotein test	–	+	–	+
	Ninhydrine test	–	+	+	–
Glycosides					
	Keller-killiani test	–	–	+	+
	Bromine water test	+	+	+	–
	Legal's test	–	+	+	+
Amino acids					
	Ninhydrine test	–	+	–	+
	Test for tyrosine	–	–	–	+
	Test for cysteine	–	–	–	–
Triterpenoids /Steroids					
	Liebermann's reaction	–	+	+	+

Wound Contraction

Excision wounds were examined for the efficacy of creams containing nano emulsions of B. ceiba extract as well as a standard ointment for 20 days. All animals except the control group were totally healed on the 20th day. Wound area measurements demonstrated that the test group's wound sizes were reduced earlier than the control group in an excision

wound model. The healing of wounds was found excellent in test and standard groups. *B. ceiba* had a significant effect in this study when compared to the control group.

Table 2. Depiction of wound healing effect of different extracts of *B. ceiba*, std. & control group rats

Days	Control	Standard	n-hexane	chloroform	Ethyl acetoacetate	Methanol
5th Day						
10th Day						
15th Day						

It can be seen clearly that wound contraction was started from the 5th day. However, the effect was maximum in methanol extract (in beginning) while wound contraction effect was better in all the rats treated with n-hexane, chloroform, and ethyl acetoacetate *B. ceiba* extracts.

At 10th day the wounds were almost recovered in all the group of rats when compared with control group. The growth of hairs was also started after wound healing. It might be assumed that wound healing was due to development of proteins.

All the groups of animals showed an excellent wound healing activity when compared with control group after 15th day of exposure with treatments. It showed a promoted cellular growth and development in all the animals.

Re-epithelization period

On 10th day after the suture was removed, re-epithelization period was examined in test, standard and control groups. Below table shows that *B. ceiba* extract had a substantial impact on wound breaking and strength recovery. The recovery was found completed by the 15th day when the animals were examined. In comparison to other groups, the best action was observed in ethyl acetoacetate extract among other solvent extracts.

In comparison to the control group, the rats treated with *B. ceiba* was entirely healed in a less time.

The control group exhibited re-epithelization period of 18.46±0.43* while standard group demonstrated the same as 7.79±0.05**. The *B. ceiba* N-hexane, chloroform, ethyl acetoacetate, and methanol demonstrated rats showed the re-epithelization period of 11.20±0.62**, 12.17±1.30***, 9.3±1.23** and 10.4±1.12***, respectively.

However, the maximum re-epithelization period was observed in ethyl acetoacetate *B. ceiba* extract as 9.3±1.23**. Therefore, it might be concluded that healing recovery was faster in the ethyl acetoacetate than other extracts.

At the conclusion of the proliferative phase, granulation tissue is mostly made up of fibroblasts, collagen, edoema, and new, small blood vessels.

The greater proliferation and differentiation of fibroblast cells into myofibroblasts may be the cause of the increased tensile strength. By applying stress to the nearby extracellular matrix and secreting extracellular matrix proteins like collagen to keep the contraction stable, myofibroblasts are thought to play a significant role in wound contraction. Collagen was shown to have an improved tensile strength in both the test and control treatment groups [13].

Table 3. Depiction of Re-epithelization period of different extracts of *B. ceiba*, std. & control group rats

Group	Re-epithelization Period
Control	18.46±0.43*
Standard	7.79±0.05**
n-hexane	11.20±0.62**
Chloroform	12.17±1.30***
Ethyl acetoacetate	9.3±1.23**
Methanol	10.4±1.12***

Significance level was denoted by *; n=6

P<0.05; readings were given in Mean± SEM

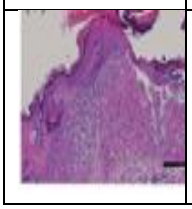
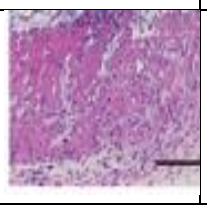
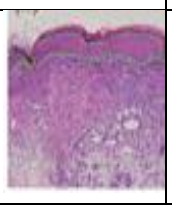
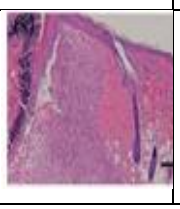
Histopathology

The wound tissues were examined for cellular infiltration and concerning pathophysiology. Compound microscope was used for the determination of the same.

After 15th day of study, the wound cells were excised to test for histopathological changes occurred in cells.

Table 5.5 shows the histopathology of different extracts- n-hexane, chloroform, ethyl acetoacetate and methanol of the *B. ceiba*. It was noted that amongst all ethyl acetoacetate extract was exhibited an excellent wound healing than other extracts and when compared with the control group.

Table 4. Histopathology of n-hexane, chloroform, ethyl acetoacetate and methanol extract of *B. ceiba*

Control	Standard	n-hexane	chloroform	Ethyl acetoacetate	Methanol
					

All wounds included fibroblast-like and inflammatory cells. In comparison to the wounds with non-ring signal, no histological trait was found to be substantially higher or lower in the wounds with ring signal. Compared to wounds with no ring signal, wounds with a ring signal typically had denatured or burst cells. Three distinct patterns of peroxidase activity staining were visible in the wounds: dense, rounded, and dispersed signals. Granular signals with a dark tint made up the dense signals.

The well-circumscribed spherical signals occasionally have intense signals inside of them. We assumed that signals that were dense and rounded meant there was intracellular peroxidase present. The top layer of the wounds, where many inflammatory cells gathered, revealed distributed peroxidase activity in the wounds with a ring signal.

After developing their nano emulsion creams to have potential against infections and their accessibility, *B. ceiba* extracts were examined for their ability to heal wounds [12]. Additionally, the creams of nano emulsions of B. ceiba as well as their combination demonstrated their ability to cure various types of wounds when compared to the traditional agent betadine. It has been demonstrated to have a less potent antibacterial impact than ginger, which has also been linked to their abilities to promote wound healing in both excisional and incisional wound models.

Cross-connecting speeds up development. The findings of this experiment unmistakably showed a beneficial effect on wound healing activities. According to theory, myofibroblasts exert pressure on the extracellular matrix and secrete ECM proteins like collagen to stabilise the contraction during wound contraction. In both the test and control treatment groups, cross-linking increased tensile strength, which promoted collagen maturation [14]. According to the results of this study [15], the secondary metabolites that give B. ceiba extracts their antibacterial and wound-healing properties may be alkaloids, saponins, terpenoids, and flavonoids. Allicin, which is present in B. ceiba extracts, is thought to help wound healing and the eradication of acne-causing bacteria [16]. In addition, it helps to improve blood circulation and reduce edoema. Ginger includes anti-oxidant and anti-inflammatory compounds such shogaol and gingerol, which have been shown to promote the growth of new blood vessels, enhancing its ability to heal wounds.

Although there is little success in current antibiotic therapy, crude extracts of these plants were efficient against multidrug resistant bacteria. This study cannot predict how these spices would affect these species in vivo. Zone as well as results must be influenced by the substance's solubility, diffusional velocity, and volatilization in agar media. All the medications shown significant wound healing abilities in both the acid burn and boiling water models during the testing for wound healing. When they examined the hydroxyproline content, wound contraction, and epithelization time, they discovered wound healing potential in all variables.

CONCLUSION

Even though all three have antibacterial and wound-healing qualities that are comparable to those of the common medication betadine, this study's comparison of the wound-healing abilities of B. ceiba revealed that ginger is more effective. According to the study, alkaloids, saponins, tannin, and flavonoids as well as other important secondary metabolites are likely to be responsible for B. ceiba's wound-healing abilities. Additionally, it was demonstrated that the suggested formulation of nanoemulsion cream was successful in achieving the greatest effect of the extracts with non-irritating application and enhanced bioavailability. Due to the presence of all of the aforementioned chemical compositions, it has been determined that the nanoemulsion formulation of B. ceiba has the strongest antibacterial and wound healing effects. The work will be useful in developing more affordable, more potent antibacterial and wound healing formulations.

It is concluded that each of these has considerably exhibited wound healing potential in various screening protocols, and that they are all beneficial in preventing bacterial development and infection. It implies that creating dosage forms and discovering and isolating the active compounds responsible for their pharmacological effect are necessary.

FUNDING
Nil.

CONFLICT OF INTEREST
None conflict of interest was declared by the authors.

REFERENCES
1. Cramer, C. S., Mandal S, Sharma S, Nourbakhsh S. S, Goldman I, Guzman I. Recent advances in onion genetic improvement." *Agronomy* 11, no. 3 (2021): pp. 482.
2. Crider, Krista S., Mario A. Cleves, Jennita Reefhuis, Robert J. Berry, Charlotte A. Hobbs, and Dale J. Hu. "Antibacterial medication use during pregnancy and risk of birth defects: National Birth Defects Prevention Study." *Archives of pediatrics & adolescent medicine* 163, no. 11 (2009): pp. 978-985.
3. Maurya Santosh Kumar, N.K. Verma, Dinesh Kumar Verm. Bombax ceiba Linn: A review of its phytochemistry and pharmacology. Current Research Journal of Pharmaceutical and Allied Sciences, July-September 2018, Vol. 2 (Issue 3), 14-23.
4. Kumar, S.N., Nambisan, B. Sundaresan, A. Mohandas, C. and Anto, R.J. "Isolation and identification of antimicrobial secondary metabolites from Bacillus cereus associated with a rhabditid entomopathogenic nematode." *Annals of microbiology* 64, no. 1 (2014): pp.209-218.
5. Kuwano, H., Yano, K., Ohno, S., Ikebe, M., Kitamura, K., Toh, Y., Mori, M. and Sugimachi, K. "Dipyridamole inhibits early wound healing in rat skin incisions." *Journal of Surgical Research* 56, no. 3 (1994): pp.267-270.
6. Leekha, Surbhi, Christine L. Terrell, and Randall S. Edson. "General principles of antimicrobial therapy." In *Mayo clinic proceedings*, vol. 86, no. 2 (2011): pp.156-167.
7. Lertcanawanichakul, Monthon, and Songtham Sawangnop. "A comparison of two methods used for measuring the antagonistic activity of Bacillus species." *Walailak Journal of Science and Technology (WJST)* 5, no. 2 (2008): pp.161-171.
8. Maali, A., and M.T. Hamed Mosavian. "Preparation and application of nanoemulsions in the last decade (2000–2010)." *Journal of dispersion science and technology* 34, no. 1 (2013): pp.92-105.
9. Manjunatha, B. K., S. M. Vidya, K. V. Rashmi, K. L. Mankani, H. J. Shilpa, and SD Jagadeesh Singh. "Evaluation of wound-healing potency of Vernonia arborea Hk." *Indian journal of pharmacology* 37, no. 4 (2005): pp.223.
10. Manzoor, A., Dar, I.H., Bhat, S.A. and Ahmad, S. "Flavonoids: health benefits and their potential use in food systems." In *Functional Food Products and Sustainable Health*, (2020): pp.235-256.
11. Manzoor, A., Dar, I.H., Bhat, S.A. and Ahmad, S. "Indigenous knowledge of medicinal plants from Gujranwala district, Pakistan." *Journal of Ethnopharmacology* 148, no. 2 (2013): pp.714-723.
12. Markowiak, Paulina, and Katarzyna Śliżewska. "Effects of probiotics, prebiotics, and synbiotics on human health." *Nutrients* 9, no. 9 (2017): pp.1021.
13. Martin, Paul. "Wound healing--aiming for perfect skin regeneration." *Science* 276, no. 5309 (1997): pp.75-81.
14. Medrado, Alena, R.A.P. Lívia, S. Pugliese, S. Regina, A. Reis, and Zilton, A. Andrade. "Influence of low level laser therapy on wound healing and its biological action upon myofibroblasts." *Lasers in surgery and medicine* 32, no. 3 (2003): pp.239-244.
15. Mekouar, Mohamed Ali. "15. Food and Agriculture Organization of the United Nations (FAO)." *Yearbook of International Environmental Law* 29 (2018): pp.448-468.
16. Morrison M, Roger D. “Desktop guide to keynotes and confirmatory symptoms” *unimedia*, (1993): pp.58-62