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Investigation on Wound Healing activity and Physiochemical Properties of Organic Bark Extract of *Commiphora wightii*(arn.) bhandari

Adinath V. Munde[1], Palanisamy Pethappachetty[2*], Maharudra S. Rakh[3], Periyasamy Parthiban[4], Anandharaj Govindaraj[5]

[1]Research Scholar, Vinayaka Mission's College of Pharmacy, Vinayaka Mission's Research Foundation (Deemed to be University), Salem, Tamilnadu- 636008, India
[2]Department of Pharmaceutics, Vinayaka Mission's College of Pharmacy, Vinayaka Mission's Research Foundation (Deemed to be University), Salem, Tamilnadu- 636008, India
[3]Department of Pharmacognosy, College of B. Pharmacy and Research Centre, Gadhi, Georai, Dist. Beed, Maharashtra- 431143, India
[4]Department of Pharmaceutical Chemistry, Vellalar College of Pharmacy, Maruthi Nagar, Thindal Post, Erode, 638012, Tamilnadu, India
[5]Department of Pharmacy Practice, Vellalar College of Pharmacy, Maruthi Nagar, Thindal Post, Erode, 638012, Tamilnadu, India
Corresponding author: adinathmunde@gmail.com mobile: +91 9423954444

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Abstract

An essential part of the traditional Indian Ayurvedic medical system was guggul. Belonging to the Burseraceae family, *Commiphora wightii* Bhandari (Syn. *Commiphora mukul Hook. ex.* Stocks). It has been used in Ayurveda since the beginning of time to treat a variety of diseases, including those with anti-inflammatory, antibacterial, anti-obesity, antioxidant, and antifungal properties. This plant is widely used in traditional Indian medicines. Based on historical usage and literary information, this plant was chosen for its potential to heal wounds. The study evaluated the ability of based on solvents bark extracts to heal wounds *in vitro*. The results of the phytochemical analysis showed that pure bark extracts contained every phytochemical component. It was discovered that the *invitro* wound healing effectiveness depended on both concentration and time. The highest level of effectiveness was observed at 100 µg/ml for a 36 h period, and the average area of the wound was recorded at 25 µg/mL during the same time frame. The current study found that, while the wound diminution area level was observed at lower concentration, the in-vitro wound healing activity result proved 100% wound healing cell migration effect at the maximum concentration at a maximum time period of 36 h. Significant wound healing activity, tissue regeneration, and inflammation regulation are found in the ethanol-based plant bark extracts. The idea that *Commiphora wightii* bark ethanolic extract exhibits crucial wound healing was also supported by histological analysis.

Keywords:*Commiphora wightii* Bhandari bark extract, Phytoconstituents, Scanning Electron Microscope, Wound healing activity.

1. Introduction

According to their source, remedies from Ayurveda are classified as either animal, mineral material or medicinal plants. According to who, 80% of people worldwide still use conventional drugs. Herbal medicine has long been utilized for illness diagnosis, treatment, and prevention.

An essential part of the traditional Indian Ayurvedic health care system was guggulu. It has been used extensively for millennia by Ayurvedic practitioners for managing a wide range of illnesses. It is also used in the fragrance and pharmaceutical industries. Guggulu, also known as *Commiphora wightii* (ARN) Bhandari, is a gum or resin derived from this tree. (Syn. Hook. ex. Commiphoramukul Stocks). A small tree or shrub in the Burseraceae family is called a guggulu. Commiphora, comprising 165 species. It is widely distributed throughout the tropical regions of Northern Africa, Madagascar, Central Asia, Australia, the Pacific Islands, India, Bangladesh, and Pakistan. It originated in Africa and Asia.

Guggulu contains volatile oil, gum resin, gugulipids, guggulsterones, guggul sterols, and other steroids. Astringent, disinfectants, expectorant, aphrodisiac, carminative, antispasmodic, and emmenagogue, guggulu is widely used in Ayurvedic medicine. According to Ayurveda, guggulu is the best herbicide for Medoroga and Vata issues. Around the world, it is also referred to as a fat burner and is frequently used to treat obesity. It helps to reduce cholesterol and triglycerides. Guggulu is especially helpful in the treatment of rheumatoid arthritis, sciatica, and gout.It is among the most significant as well. Guggulu is a vital and dependable herb in Ayurvedic medicine according to rasayana. It has been used for the management of rheumatism, obesity, gout, inflammation, arthritis, and issues related to lipid metabolism since ancient times. Since *Commiphora wightii* is a plant that is widely used in healthcare and nutraceutical products, the goal of this study was to examine the plant's cell the rate of migration and wound healing efficacious metabolic signature. All the organic extracts from bark have been investigated using infrared spectroscopy and SEM (Scanning electron microscopy) analysis in order to achieve this goal. Ethanolic extracts *in vitro* wound healing assay was evaluated (Parasuraman*et. al.,* 2014, Oziom*aet. al.*,2019, Shishodia*et. al.*,2008, Bhardwaj*et. al.*,2019, Singh*et. al.*,2015, Barve Vaibhavi*et. al.*,2013, Kumar*et. al.*,2020).

1.1. Plant outline

The plant that produces flowers *Commiphora wightii*, also known as the Indian bdellium-tree, gugal, or mukul myrrh tree, relates to the *Burseraceae* family and yields a smells resin that can be utilized in ayurvedic medicine and in incense. The species is farmed in western India and southern Pakistan. It was tolerant of poor soil and primarily prefers arid and semi-arid

climates. The small tree *Commiphora wightii* (Fig 01) can reach a maximum height of 13 feet and has papery-colored bark. Simple or trifoliate leaves with ovate leaflets that are 1-3 cm (0.39-1.97 inch) long, 0.5-2.5 cm (0.20-0.98 inch) wide, and irregularly toothed are found on the leaves. It is gynodioecious; some plants have male and bisexual flowers, while others only have female flowers. Each flower has four small petals and ranges in colour from red to pink. When ripe, the tiny, spherical fruits turn red.

Figure 01: *Commiphora wightii,*

2. Materials and Methods

2.1. Collection and Extraction

The plant evidence was collected in the environs of Salem, Tamil Nadu. Dr. Mutheeswaran, a scientist at the Xavier Research Foundation in Tirunelveli, Tamil Nadu, India, identified the plant as *Commiphora wightii*. After the *Commiphora wightii* bark was taken off the gathered branches, it was thoroughly cleaned with ordinary tap water to remove any impurities. After that, the bark were given one more washing in Millipore water and left out on a clean floor in the shade for three weeks to air dry. First, a coarse powder was made from the crop material, and then a fine powder.

Commiphora wightii Bhandari (250g) was obtained for 72 h at 60–80°C using petroleum ether, chloroform, acetone, and ethanol as solvents. After extraction, any potential impurities were removed by passing the defatted extracts through Whatman filter paper (No. 10) while they were still hot. The extract was reduced in volume to 1/10 using vacuum distillation; the resulting concentrated extract was then moved to a 100 mL beaker, and the leftover solvent was removed in a water bath. The extract had a color that was dark brownish. Subsequently, the highly concentrated extract was dried out by placing it in a desiccator. The extract that was dried was kept in a glass container that was airtight for later study (Evens *et. al.,* 2002, Aher *et. al.,* 2012, Li*et. al.,* 2011).

2.2. Identification of Phytochemical Active Constituents

The extracted materials, which included petroleum ether, chloroform, acetone, ethanol, and aqueous solvents, were subjected to various tests to detect alkaloids, flavonoids, carbohydrates, glycosides, amino acids, saponins, tannins, phenolic compounds, terpenoids, and steroids (Kokate *et. al.,* 2003,Irudayaraj*et. al.*,2010, Harborne*et. al.*,1998, Roghini*et. al.*,2018, Usman*et. al.*,2009).The subsequent reports are displayed in Table 01.

2.3. Analytical screening of Physico-chemical properties

2.3.1. ATR- FTIR Spectra analysis: The spectrum of wavelengths of 4000 to 400cm^{-1} was used to record the spectrum. An ATR-FTIR (Attenuated total reflectance - Fourier transform infrared) spectrophotometer was utilized to acquire an infrared spectrum subsequent to the prompt insertion of a medication sample into the sample holder cavity (Finlayson*et. al.*,2022, Montesdeoca*et. al.*,2022). By including excipients in the ethanolic extracts, peaks can be identified. The reports displayed in Fig 02–07.

2.3.2. Particle size (SEM Analysis): An electron beam is used in the scanning electron microscope test method to scan a sample and produce an enhanced view for analysis. The method is frequently used in a microanalysis and failure investigation of solid inorganic materials and is also referred to as SEM analysis and SEM microscopy. High magnification electron microscopy accurately quantifies minuscule features and objects and generates high-resolution images (Knott*et. al.*,2008, Vernon*et. al.*,2000). The reports that are displayed in Fig. 08.

2.3.3. *In-vitro*wound healing assay: Scrape the cell a single-layer in a straight line by employing a 200L pipette tip. To get rid of the debris and uniform the outermost part of the scratch, wash the cells once with 1 milliliter of the growth medium. Then, add 5 milliliters of fresh medium. Important action: It is important to make scratches in cells serving as controls and the cells under assessment that are approximately the same dimensions in Table 02 and Fig 09 in order to minimize any potential variance caused by the variation in scratch width.

2.3.4. Critical step: (i)It is essential to make scratches in the examined cells as well as regulate cells that are roughly the same size in order to minimize any potential variance caused by the variation in scratch width (Liang*et. al.*,2007).

(ii) In order to capture the same field when capturing the image, make marks that will act as reference points close to the scratch. To provide the reference points, the well plate can be carefully etched on the outside of the dish with a razor blade or labelled with an ultrafine tip marker. After creating the reference points, place the dish according to a phase-contrast microscope, making sure the reference mark is inside the objective's field of perception but outside of the recorded picture field. Acquire the original photo of the scuff.

(iii) The well plate needs to be stored at 37 °C in a tissue culture incubator. Photomicrographs were taken over a variety over time intervals (0 h, 12 h, 24 h, and 36 h). The incubation period must be empirically determined for the particular cell type being used. The well plates can be taken out of the incubator and occasionally examined before being put back in to complete the incubation process (Cory*et. al.*,2011).

2.3.5. Critical step: Even if the cells are moving quickly, choose an incubation period that will enable them to heal the scratch completely. After the incubation period, place the dish according to a phase-contrast microscope, align the was photographed region from Step 6 with the centre of the reference point, and take another image. Similarly, photos need to be taken until the wound is completely healed (Muniandy 2018).

2.3.6. Cell Migration rate and Average wound area: The images were examined using American "ImageJ" software to ascertain the migration rate. After that, a percentage of the resolved area was computed and contrasted with the result obtained at 0 h. An increase in the percentage of the closed area suggested cell migration. Consequently, this implies that the healing of wounds is successful (Glaß *et. al.,* 2012, Fronza *et. al.,* 2009). The reports displayed in Table 03 and Figure 11.

3. Results

The natural phytoconstituents were identified using the accepted quality tests. As indicated in Table 01, *Commiphora wightii* bark extracts contained maximum natural chemical compounds.

Table 01: Phytochemical studies of bark extracts of *Commiphora wightii* (ARN) Bhandari

S.no	Constituents	Tests	Pet. Ether	Chloroform	Acetone	Ethanol	Aqueous
1	Alkaloids	Dragendorff's test	-	+	+	-	-
		Hager's test	+	+	-	-	+
		Wagner's test	+	-	-	+	-
		Mayer's test	-	+	-	-	+
2	Carbohydrates	Anthrone test	-	+	+	+	-
		Benedict's test	-	+	+	-	-
		Molisch's test	-	+	+	+	+
		Fehling's test	-	+	-	-	+
3	Flavonoids	Shinoda's test	+	-	+	+	-
		With conc. sulphuric acid test	+	+	+	+	+
4	Glycosides	Molisch's test	+	-	+	+	-

5	Amino acid	Million's reagent	+	-	-	+	+
		Ninhydrin reagent	+	-	+	+	-
		Biuret test	+	+	-	-	+
6	Saponins	Foam test	-	+	-	-	-
7	Tannin	Test with ferric chloride	-	-	+	-	-
8	Phenolic compounds	Test with NaOH	+	+	-	-	+
		Test with lead acetate solution	+	-	-	+	+
9	Terpenoids	Salkowski test	+	-	+	+	+
10	Steroids	Liebermann - Burchard's test	+	+	+	+	+

3.1. ATR- FTIR Spectra analysis

The ATR-FTIR compatibility study for ethanolic bark excipients revealed no interactions between the excipients and the extract reports displayed in Fig 02-07. Following SEM analysis, as illustrated in Fig 08, infrared spectroscopy studies show that no additional peaks were seen in any of the excipients, and they concluded that all of the excipients are incompatible with ethanolic extract.

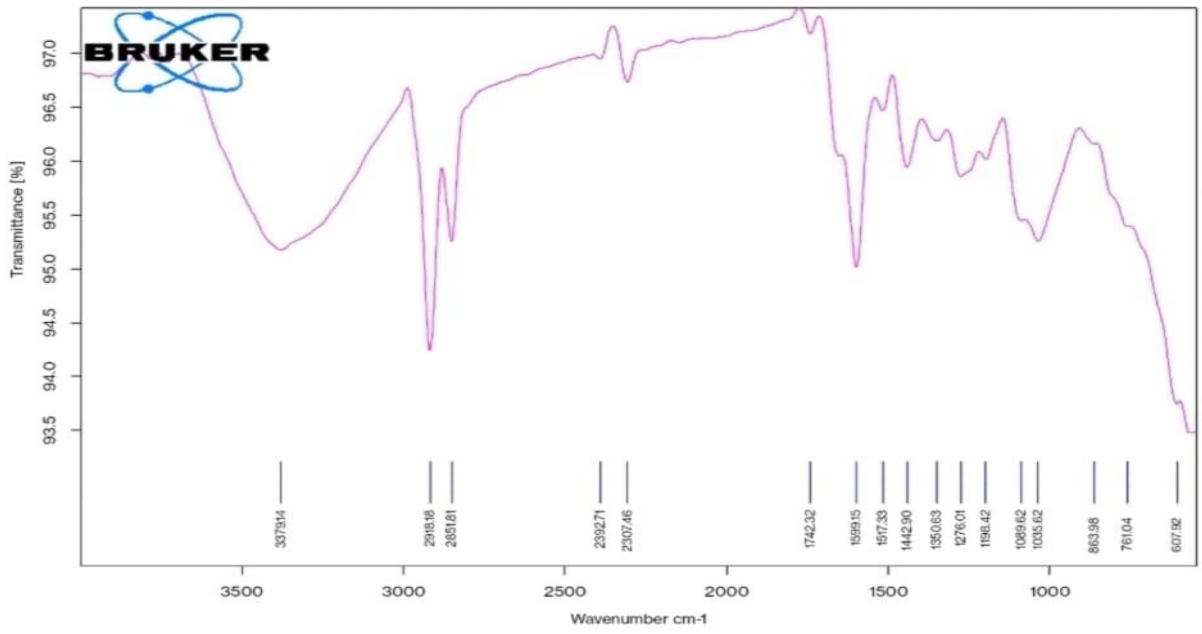

Figure 02: ATR-FTIR Spectrum of ethanolic bark extract of *Commiphora wightii* (ARN) Bhandari

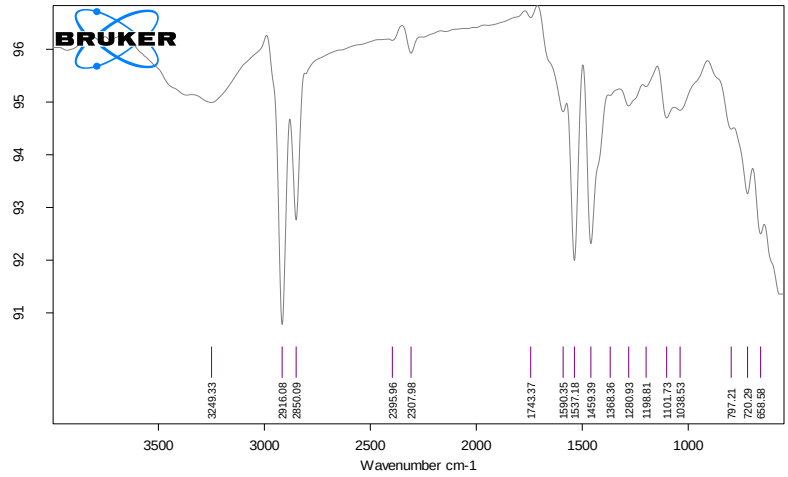

Figure 03: ATR- FTIR Spectrum of ethanolic bark extract of *Commiphora wightii* extract + Carbopol 934

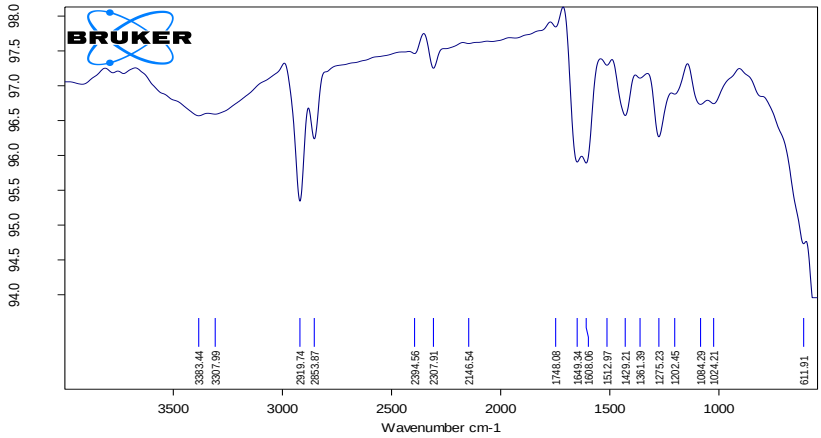

Figure 04: ATR- FTIR Spectrum of ethanolic bark extract of *Commiphora wightii* extract +Propylene glycol

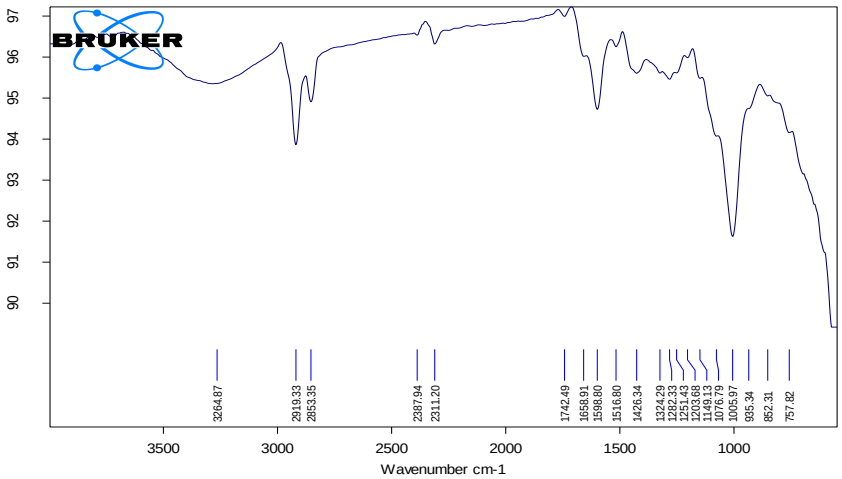

Figure 05: ATR- FTIR Spectrum of ethanolic bark extract of *Commiphora wightii* extract +Methyl paraben

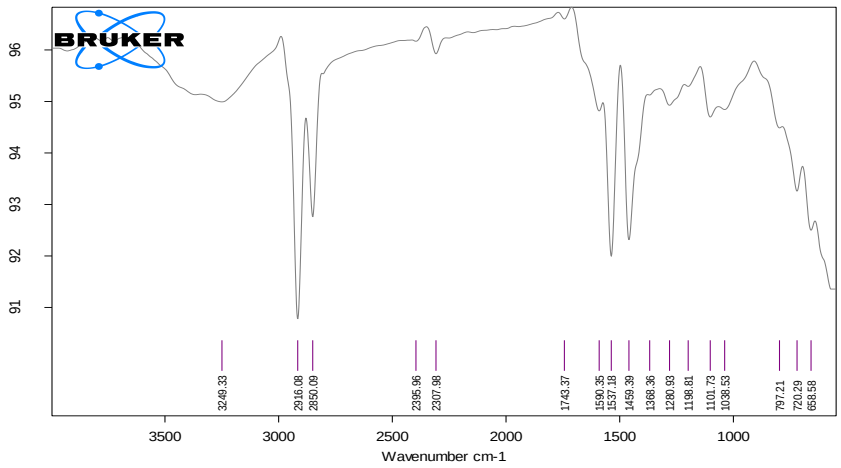

Figure 06: ATR- FTIR Spectrum of ethanolic bark extract of *Commiphora wightii* extract + Triethanolamine

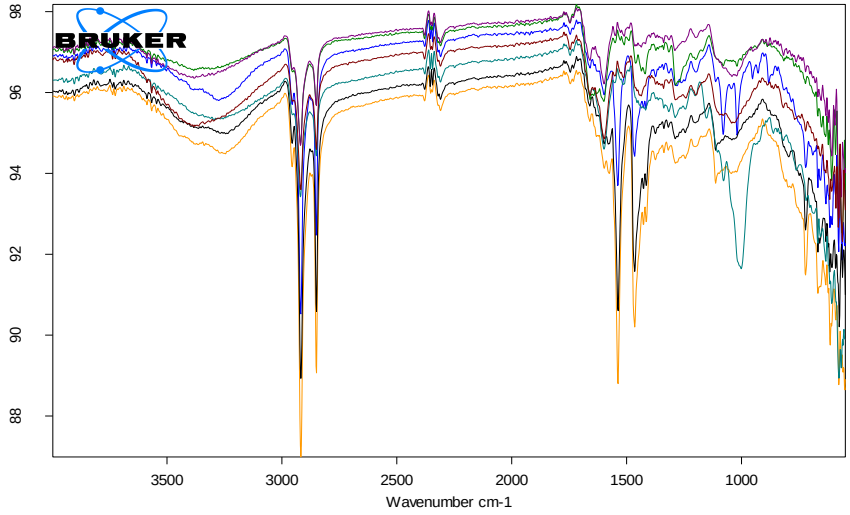

Figure 07: ATR- FTIR Spectrum of ethanolic bark extract of *Commiphora wightii* extract + All excipients

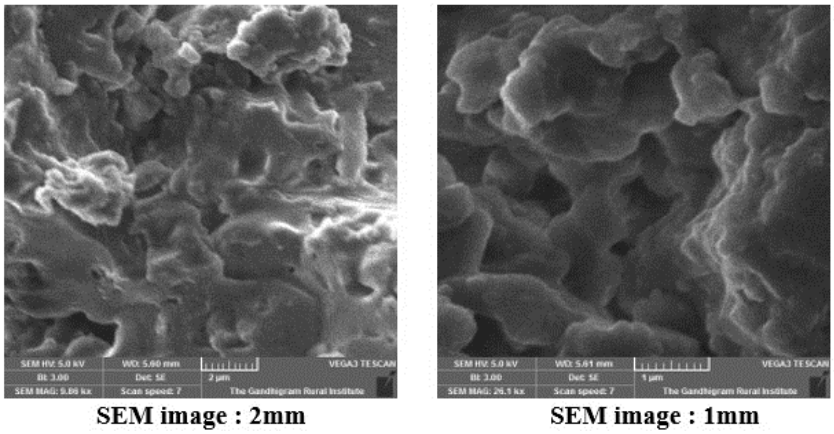

Figure 08: Particle size of SEM Analysis image on ethanolic bark extract of *Commiphora wightii* (ARN) Bhandari

3.2. Invitro-wound healing

It was discovered that the effectiveness of wound healing depended on both concentration and time. The representative photomicrographs demonstrated the considerable wound healing efficacy elicited by the ethanolic sample. As illustrated in Fig 09, the concentration of 100 µg/mL produced the maximum efficacy over a 36h period, while the average surface of the wound was recorded at 25 µg/mL during the same time frame. This suggests that the sample can be utilized on human cells even when there is evidence of is scratching or wounds and that it can be successfully developed for medical purposes as a wound healing agent.

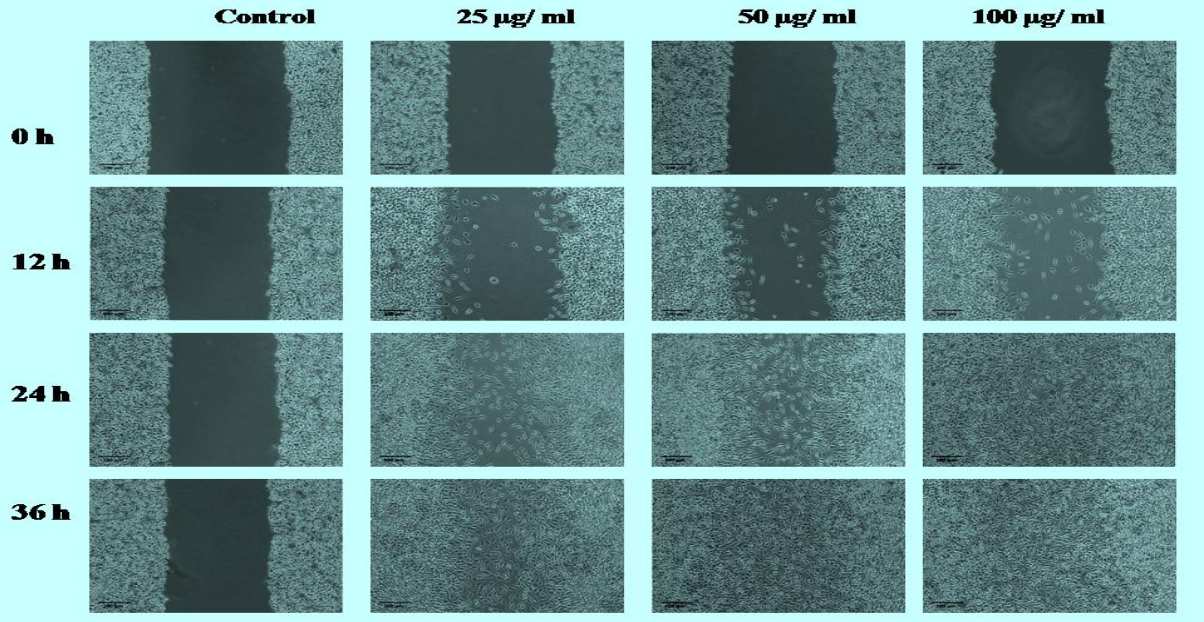

Figure 09: *In vitro* wound healing activities of ethanolic bark extract of *Commiphora wightii*

Table 02: Average Wound area of ethanolic bark extract of *Commiphora wightii* (ARN) Bhandari

Sample (µg/ml)	Average Wound area (Arbitrary units)			
	0 hours	12 hours	24 hours	36 hours
Control	75	73.1	71.5	69.4
25	75	67	62	40
50	75	44	21	7
100	75	34	10	0

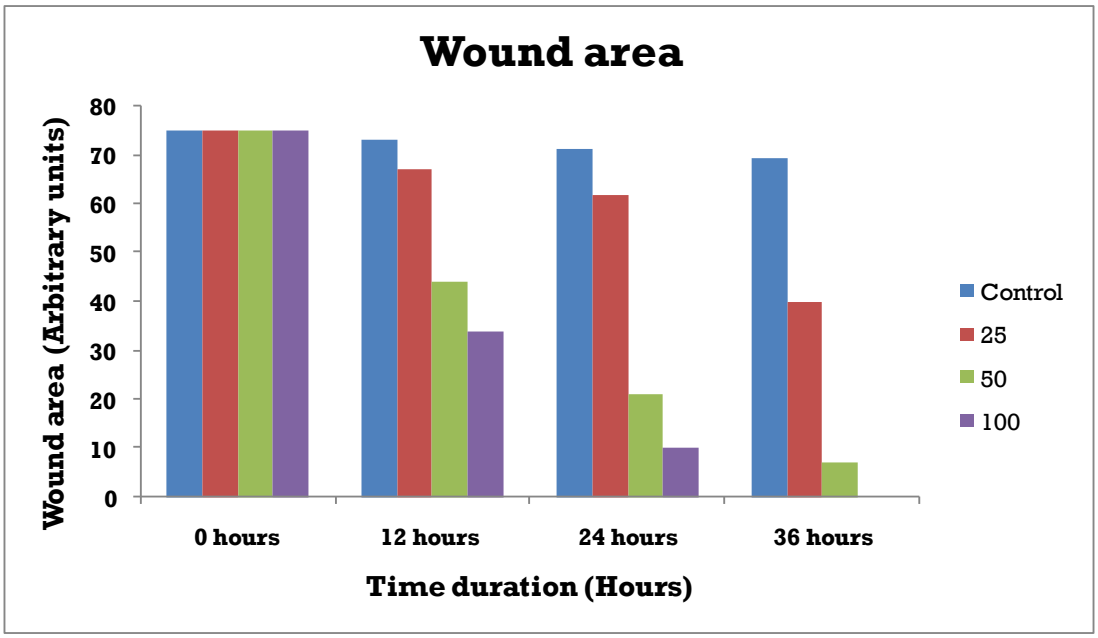

Figure 10: Graphical Representation average wound area of ethanolic bark extract of *Commiphora wightii* (ARN) Bhandari

Table 03: Cell migration rate of ethanolic bark extract of *Commiphora wightii* (ARN) Bhandari

Sample (µg/ml)	Cell Migration Rate (%)			
	0 hours	12 hours	24 hours	36 hours
Control	0	2.53	4.67	7.47
25	0	10.67	17.33	46.67
50	0	41.33	72.00	90.67
100	0	54.67	86.67	100

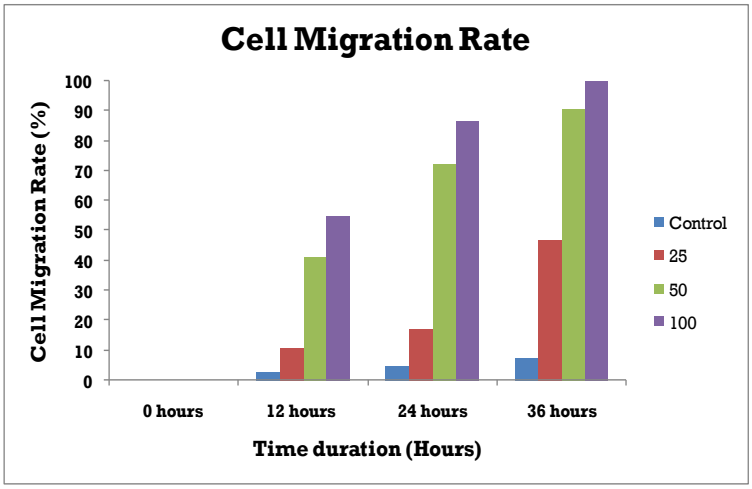

Figure 11: Graphical Representation cell migration rate of ethanolic bark extract of *Commiphora wightii* (ARN) Bhandari

4. Discussion

Through a variety of chemical tests, the plant constituents of extracts from bark were validated for our current study. The ethanolic bark extract of *Commiphora wightii* Bhandari and its excipients was subjected to an ATR-FTIR spectrum. The results indicated the disappearance of existing peaks and any additional new peaks were not clearly visible in the spectra. This suggests that the pure extract and the excipients that make it up do not interact in any way. The surface morphology was assessed using a scanning electron microscope. The results, which are displayed in Fig 08, explain that the particles were spherical in shape and randomly distributed, with sizes ranging from 1 to 2 mm. The reduction of the wound area was measured in a concentration and timedependent manner. Table 03 displays the increased cell migration rate, which is indicative of improved wound healing.The highest level of effectiveness was attained by increasing the concentration from 25 to 100 µg/mL. The sample showed a cell migration rate of 100 µg/mL within the same hour, and the maximum average efficacy reduction of the wound area was 25 µg/mL in 36 h.

5. Conclusion

The findings of our study led us to the conclusion that traditional herbal medicine methods have been successful in disease diagnosis, prevention, and treatment. Antiseptics, topical antibiotics, and oral antibiotics can increase the risk of developing Methicillin-resistant *Staphylococcus aureus* (MRSA) and other forms of antimicrobial resistance. Using a Soxhlet apparatus, the bark of *Commiphora wightii* Bhandari was extracted ethanolically, and the resulting extract was examined for these and other investigations. The ethanol production bark extracts demonstrated the ability to heal wounds *in vitro* and to regenerate tissues while controlling inflammation.

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7. Declaration/Conflict of interest

The authors report no conflicts of interest to declare in connection with this manuscript

8. Funding

There is no fund used for our research work.

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