

WOUND HEALING AND ANTIOXIDANT ACTIVITY OF METHANOLIC LEAVES EXTRACT OF MELISSA OFFICINALIS AND STREBLUS ASPER

Jyoti Verma^{1*}, Prakash Deep²

^{1,2} Maharishi School of Pharmaceutical Sciences, Maharishi University of Information Technology, IIM Road, Lucknow 226013, Uttar Pradesh, INDIA

*Email: jyotimph9@gmail.com

ABSTRACT

The current research was aimed at assessing the wound healing potential of the methanolic leaf extracts of *Melissa officinalis* and *Streblus asper* by employing the excisional wound model in albino Wistar rats. The methanolic extracts were obtained by Soxhlet extraction to facilitate effective recovery of phytoconstituents and then formulated as topical ointments at 5% and 10% concentration employing a compatible ointment base. These preparations were applied topically to full-thickness excisional wounds, and their therapeutic potential for wound healing was compared with a control treatment group given soframycin ointment. Wound contraction rate, epithelialization time, and histopathological structure of the tissue formed during healing were measured to assess the therapeutic activity. Histological analysis of wound tissue showed increased re-epithelialization, fibroblast proliferation, neovascularization, and collagen deposition in extract-treated groups, reflecting active regeneration of tissue. From the formulations so evaluated, 10% methanolic ointment of *Melissa officinalis* exhibited the maximum percentage of contraction of wound (97.84%) on the 14th day, significantly greater than that by the corresponding *Streblus asper* formulation (96.41%) ($p < 0.01$). Equivalently, the 5% *Melissa officinalis* cream showed a contraction rate of 95.72%, slightly greater than that in the 5% *Streblus asper* group (94.56%). These results indicate that the two plant extracts have significant wound healing activity, which may be due to their bioactive phytochemicals like flavonoids, tannins, and phenolic compounds having been proven to accelerate tissue repair by causing antioxidant, anti-inflammatory, and antimicrobial effects.

KEYWORDS: Wound healing, epithelization, *Streblus asper*, *Melissa officinalis*

INTRODUCTION

The foundation of the healthcare system remains natural items, which also provide both conventional and synthetic herbal treatment¹. Because plants contain a variety of life-sustaining components, scientists are looking at how these plants might be used to heal chronic wound and some infectious disorders. One of the main barriers to the development of bacterial pathogen infections in interior tissues is physical disturbance of the skin, which results in wounds. This disruption causes the cellular, anatomical, or functional continuity of live tissue to be lost or broken². The three phases of wound healing include remodeling, proliferation, and inflammation. The proliferative phase includes epithelialization, granulation tissue formation, angiogenesis, collagen deposition, and wound contraction. Endothelial cells create new blood vessels through a process called angiogenesis. Fibroblasts multiply and release collagen and fibronectin to form a new, transient extracellular matrix during the development of granulation tissue and fibroplasia. Elevated hydroxyproline levels, a biochemical indicator of tissue collagen, are found in collagen, the main substance that supports and reinforces extracellular tissue³. Epithelialization is the process by which epithelial cells proliferate and spread throughout the wound's surface. The wound contracts in tandem with myofibroblast contraction. The release of growth factors and other cytokines by platelets. Chronic wounds are those that do not heal even after receiving adequate and appropriate care. Managing such wounds can be challenging and frustrating. Current treatments for chronic wounds include debridement, irrigation, tissue grafts, antibiotics, and protease enzymes; however, these methods have serious drawbacks and unexpected complications. We have assessed the ability of methanolic extracted leaves of *Streblus asper* and *Melissa officinalis* to promote wound healing^{4,5}.

Within the Lamiaceae family, *Melissa officinalis* L., or lemon balm, is one of the most well-known and traditional medicinal plants. Although it originated in the eastern Mediterranean region, It is today widely grown in both the United States and Europe. Aromatic dried leaves, previously used for their medicinal properties, have antispasmodic and antiviral properties⁶, with no unwanted effects to date. Its alcoholic leaf extracts that contain phenolic acids (caffeic, chlorogenic, and rosmarinic acids), flavonoids (luteolin, kaempferol, and quercetin), and volatile monoterpenes (citral and citronellal) are responsible for different biological activities like antiviral, antioxidant, antibacterial, anticholinesterase, and antiproliferative activities^{7,8}. The development of α -glucosidase

inhibitors and antibacterial agents from lemon balm leaf extracts is particularly significant for the treatment of infectious diseases and metabolic conditions like diabetes and obesity^{9,10}.

One such plant is the *Streblus asper* Lour from the family Moraceae. The plant is indigenous to India, Philippines, Malaysia, Thailand and Sri Lanka. The plant is believed to have antimicrobial, anti-inflammatory, antioxidant, anti-pyretic, anti-plaque, activities and also used in diarrhea and dysentery.^{11,12,13} The plant is vastly used in India mostly in the North Eastern states of the country. The local people use the plant as dental sticks to brush the teeth and it helps to get rid of dental problems and periodontal problems. In Assam the plant is called as 'sora' and people believe that the use of this as a dental stick can prevent all the ailments related to teeth and from ancient time it has been a reason for the long lasting teeth for the aged people in the state. The scientists have found that the plant is very rich in cardiac glycosides, triterpinoids as well as phytosterols¹⁴. The tree has a number of uses. In Thailand it is one of the potential sources for paper making. *Streblus asper* is a multipurpose plant that is primarily found in Vietnam. Its edible fruits are tasty, and it also produces leaves that can be used for cleaning and tea, medicinal seeds, animal feed, valuable timber, and ornamental potential.

MATERIAL AND METHODS

Collection and authentication of plant material: In the month of February, fresh leaves of the plants *Melissa officinalis* and *Streblus asper* were collected from Mphmi Nursary in Delhi NCR and Kadipur town in the Sultanpur Local Area, respectively. NIScPR/RHMD/Consult/2023/4373-74-1 and NIScPR/ RHMD/Consult/2023/4373-74-2 are the authentication numbers for the plant material that was identified and confirmed by a taxonomist at the CSIR-National Institute of Scientific and Industrial Research in Delhi.

Extraction: Samples of plants were cleaned with water, allowed to air dry for seven days at room temperature, and then oven-dried at 40°C to eliminate any remaining moisture. The dehydrated leaves were ground up and put away for later use in an airtight container. The leaves were shade-dried, then crashed into a powder and stored in an airtight container until you needed them again.

Approximately 200 g of each plant's leaves were extracted separately with 80% methanol using Soxhlet equipment until full extraction. The extracts were subjected to preliminary phytochemical screening a dark green 10 g concentrate extract was obtained from the process¹⁴.

GC-MS Analysis: GC-MS was used to analyze the methanolic extract of *Streblus asper* and *Melissa officinalis*. The RTX-5MS capillary column is easily accepted by the GC system (PerkinElmer Clarus 600). A steady flow rate of 1.0 mL/min was achieved and maintained using 99.99 percent pure helium. The GC-MS spectral lines were identified using an ionization energy technique with an ionization energy of 70 eV (electron volt), a scantime of 0.2 seconds, a fragment extending from 40 to 650 m/z, an injection amount of 1 L (split ratio 10:1), and a temperature of 260 °C. The column oven, on the other hand, ran for three minutes.

The components were identified by comparing the plant samples' retention times, peak areas, peak heights, and spectral line patterns to those of known chemicals in databases from the National Institute of Standards and Technology (NIST) library and the Wiley-8 library.^{6,11}

DPPH Scavenging Assay: 0.2 ml of 0.1 mM DPPH solution (SRL Chem, Cat no. SR-29128) in methanol (SD fine, Cat no. 109301C250) was combined with 10 µl of the test material from a different stock. Ascorbic acid (SRL, Cat no. 23006) served as the standard. The reaction was set up in quadruplicate, and 0.2 ml of DPPH and 10 µl of standard/sample at different concentrations were used to create duplicate blanks. Red-controlled wells were those that lacked the reagent (DPPH). For 30 minutes, the plate was kept in the dark. At the end of the incubation, the decolorization was detected at 517 nm using a microplate reader (iMark, BioRad). A reaction mixture containing 20 µl of deionized water served as the control. The scavenging activity was shown as a percentage of inhibition relative to the control. The IC₅₀ was calculated using Graph Pad Prism 6 software. The X (sample concentration) and Y (inhibition % relative to control) axes were used to construct a graph.

$\% \text{RSA} = (\text{ABS control} - \text{Abs Sample}) / \text{Abs control} \times 100$

where RSA=Radical Scavenging Activity, ABS Control=Absorbance of control and Abs Sample Absorbance of Sample.

Preparations of ointment

Preparation of Extract-Based and Straight Ointment Bases: The plain ointment base was prepared according to the British Pharmacopoeia (BP, 1988a) specifications. Wool fat (10 g), cetostearyl alcohol (10 g), hard paraffin (10 g), and white soft paraffin (170 g) were the ingredients.

The ingredients were weighed accurately, dried with a powder dry capsule, mixed thoroughly, and gradually heated under continuous stirring until an homogeneous molten mass resulted. The molten mass was permitted to cool at room temperature, yielding a smooth and homogenous ointment base.

For the making of extract-based ointments, two strengths—5% w/w and 10% w/w—were prepared, each containing a total of 50 g weight. To make the 5%, 2.5 g of methanolic crude extract of either *Melissa officinalis*

or *Streblus asper* were added to the simple ointment base (47.5g). For the 10%, 5 g of methanolic crude extract was mixed with the simple ointment base (45 g). The mixing was carried out until a uniform and homogenous product was obtained. The 5% w/w soframycin ointment was the extract-based treatments' relative efficacy for wound healing using the positive control.

The plain ointment base without an active extract was the negative control. Sterile, airtight containers were stored with all the ointments to provide stability and for hygienic topical application during the experimental duration ¹⁵.

Experimental Animals: Healthy Wistar albino rats of 150–200 g were obtained from the Animal House complex of ICAR–Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh (243122), India. Standard laboratory conditions, including a 12-hour light/dark cycle, a regulated temperature ($25 \pm 1^\circ\text{C}$), and a relative humidity of $55 \pm 5\%$, were maintained for the animals. They were provided free access to standard pellet food (Shree Balaji Agro Industries, Lucknow) and clean drinking water. All experimental protocols were followed according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and prior approval was taken from the Institutional Animal Ethics Committee (IAEC).

Experimental Design and Group Allocation: The potential for wound healing of the produced formulations was examined using an excisional wound model.

A total of 35 rats were randomly assigned into seven groups ($n = 5$ per group) as follows:

Group I: Diseased control (no treatment)

Group II: Negative control group (simple ointment base treatment)

Group III: Positive control group (5% soframycin ointment treatment)

Group IV: Test group (5% *M. officinalis* ointment treatment)

Group V: Test group (10% *M. officinalis* ointment treated)

Group VI: Test group (5% *S. asper* ointment treated)

Group VII: Test group (10% *S. asper* ointment treated) ^{16,17}.

Extensional Wound Model Procedure: Rats were anesthetized with intraperitoneal ketamine (80 mg/kg) and xylazine (5 mg/kg). The dorsal skin was shaved off using an electric trimmer, and the site disinfected with 70% ethanol or an iodine antiseptic solution. An 8 mm diameter full-thickness excisional wound was made using a sterile biopsy punch. Immediately after surgery, animals were individually caged to reduce self-inflicted interference with the wound site.

Topical treatment with the respective ointments was started 24 hours after wounding and repeated once daily until full epithelialization. The course of wound healing was followed over a period of 14 days by macroscopic inspection, planimetric determination of the wound area, and histopathologic investigation of the wound tissue. Measurement of wound area and contractions (%)

The measurement of the wound area on days 4, 8, 12, and 16 by using transparent graph paper and digital caliper. Wound contraction % was calculated by following formula:

$$\% \text{ closure} = \frac{\text{wound area on Zero day} - \text{Wound area on corresponding day}}{\text{Wound area on Zero day}} \times 100$$

This enabled quantitative evaluation of wound closure rate with time. Measurement of Epithelialization Period

The epithelialization period was measured as the duration in days for scab removal without raw wound. This parameter was used as an indirect estimation of tissue regeneration and re-epithelialization.

Histopathological Evaluation: Specimens of granulation tissue biopsy obtained on day 14 were preserved for 24 hours in 10% neutral buffered formalin. The samples underwent dehydration using graded ethanol and cleared with xylene series of solutions ^{19,20}. They were later embedded in paraffin wax (melt point $40\text{--}60^\circ\text{C}$), and a microtome was used to cut slices that were 10 μm thick. Hematoxylin and eosin (H&E) was used to stain the sections under microscopic examination. Histopathological parameters measured were inflammatory cell infiltration, fibroblast proliferation, collagen deposition, and neovascularization.

Statistical analysis

Every value was shown as mean $\pm$ SEM. Dunnett's multiple comparison test was used after a one-way ANOVA for statistical analysis. Using a p -value < 0.05 , the significance was established.

RESULTS

Identification of components: Identification was done using the calculated fragments, molecular mass and molecular structure. The NIST database and the Willy 8-library, which has more than 60,000 pattern entries, were used to interpret the GC-MS spectra. Together with their molecular weights and chemical structures, the components of the test materials were determined. The percentage amounts discovered in the sample were contrasted with component spectra from the Willy 8 collection and the NIST library. This was done in order to determine whether this plant species has any chemicals or a combination of them that could support its traditional and commercial therapeutic purposes. It also helps to determine the most effective methods for removing harmful compounds. The test materials constituent parts were determined (Table 1, 2).

Table-1: Compound of Methanolic leaves extract of *Melissa officinalis*

S.N	RT	Compound	%Area
1	10.22	Citral	21.37
2	13.85	Geraniol	16.80
3	16.43	Caryophyllene	11.63
4	18.50	2,4-Cresotaldehyde	1.18
5	19.70	Rosmarinic acid derivative	9.88
6	21.34	Dodecanoic acid	0.58
7	22.55	Linalool	6.42
8	24.78	Tetradecanoic acid	1.43
9	26.35	2,6,10-Trimethyl-14-ethylene-14-pentadecene	0.48
10	28.11	Hexadecanoic acid, methyl ester	8.22
11	29.07	5-(Hydroxymethyl)-2-furan carboxaldehyde	29.04
12	33.42	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	51.55
13	33.78	Octadecanoic acid	3.23
14	34.05	Malonic acid, 3-hexyl tridecyl ester	0.38
15	40.34	1,2-Benzenedicarboxylic acid	0.34
16	50.21	Tocopherol (Vitamin E)	0.39
17	55.20	Stigmast-5-en-3-ol (β -Sitosterol)	1.13

Table-2: Compound of Methanolic leaves extract of *Streblus asper*

1	7.28	Glycerol	1.02
2	9.45	Benzaldehyde	0.89
3	11.24	Butanedioic acid (Succinic acid)	1.45
4	12.25	Hexadecanoic acid (Palmitic acid)	18.27
5	15.34	Phytol	11.86
6	18.63	Pentadecanoic acid	2.12
7	20.50	n-Hexadecanoic acid (Palmitic acid)	42.76
8	21.29	Squalene	6.78
9	21.76	Heptadecanoic acid	1.50
10	22.41	9,12-Octadecadienoic acid (Linoleic acid)	7.44
11	24.07	Octadecanoic acid	5.87
12	25.87	Phytol	3.55
13	31.40	β -Sitosterol	5.38
14	34.67	Lupeol	4.55
15	36.12	α -Amyrin	2.6

Numerous chemicals were found in the methanolic extract MO and SA samples during GCMS analysis. Seventeen compounds were found in samples with MO and fifteen compounds in samples with SA, already included in the tables.

Antioxidant activity (DPPH assay)

Melissa officinalis leaf extract exhibited significantly higher antioxidant activity ($IC_{50} = 46.4 \mu\text{g/mL}$) compared to *Streblus asper* ($IC_{50} = 74.8 \mu\text{g/mL}$), which correlates with its higher content of phenolic and terpenoid compounds identified by GC-MS.

Table-3: DPPH Scavenging Assay

Sample	IC50Value ($\mu\text{g/ml}$) (Mean $\pm$ SEM)
MO	213.6 $\pm$ 0.03
SA	575.06 $\pm$ 0.06
Ascorbic Acid	11.95 $\pm$ 0.02

Wound Healing and Contraction: 5% w/w and 10% w/w ointments of methanolic extracts of *Melissa officinalis* and *Streblus asper* showed considerably improved wound healing activity than the negative control group. Specifically, it was noted that from day 4 to day 14 ($p < 0.001$), the 10% extract ointments demonstrated a notable shrinkage of the wound. Significant contraction was observed for 5% *S. asper* extract from day

6, while 5% *M. officinalis* extract revealed significant contraction from day 4 onwards.

The maximum contraction of the wound was seen in the 10% *S. asper* group with 96.42 % closure on day 14, followed by the 10% *M. officinalis* group with 97.84% closure. Soframycin also indicated significant contraction of the wound from day 2 ($p < 0.01$) and continued to do so until day 14 ($p < 0.001$).

There was a significant decrease in epithelialization time in all treated groups when compared with the diseased control. The shortest epithelialization time (approximately 4 days) was reported from the 10% extract-treated groups, especially those treated with *M. officinalis*, which showed enhanced tissue regeneration and surface healing ($p < 0.001$).(Table1)

Period for epithelization: A marked decrease in the time of epithelialization was noted in all treatment groups, viz., standard (soframycin) and both percentages of the methanolic extracts, in contrast to the control group. Interestingly, the 10% methanolic leaf extract-treated groups showed the most vigorous effect, which had complete epithelialization as early as the 4th day post-treatment, statistically significant ($p < 0.001$) when compared with the untreated control group. This rapid re-epithelialization reflects increased cellular migration and proliferation, presumably mediated by the bioactive phytoconstituents' presence that is able to induce keratinocyte activity, suppress local inflammation, and activate fibroblast function critical processes during wound remodeling and tissue repair.(Figure1, Figure 2).

Histopathological Findings: Microscopic analysis of the granulation tissue validated the therapeutic effect of the extracts. In the 10% *M. officinalis* ointment treatment group, the tissue displayed dense collagen fiber deposition, rich fibroblast proliferation, lack of inflammatory cells, and minimal angiogenesis implying advanced healing.

The 5% *S. asper* and 5% *M. officinalis* extract groups indicated moderate collagen organization, decreased inflammatory response, and moderate fibroblast growth. The soframycin-treated group exhibited mild fibroblast activity and less collagen content than 10% extract-treated groups.

On the contrary, tissues from the negative and diseased control groups presented rare collagen fibers, weak fibroblast activity, lack of new blood vessels, and severe inflammatory infiltration, reflecting delayed and incomplete healing of wounds.

Table 4. The excisions wound healing model's wound area (mm²) and percentage of wound contraction

Drug Treatment	Diseased	Base Control	Standard	5% SA	10% SA	5% MO	10% MO
2 nd day	71.628±0.32 (10.04)	169±1.6 (10.428)	466±2.0 (24.214)	236±1.0 (21.282)	372±1.6 (23.58)	153±0.68 (21.78)	192±0.85 (31.357)
4 th day	432.36±1.9 (22.69)	552±5.5 (21.834)	397±1.7** (42.034)	307±1.3 (37.574)	294±1.3** (42.31)	269±1.2* (40.91)	429±1.9** (46.678)
6 th day	560.748±2.5 (35.76)	639±6.3 (30.656)	300±1.3 (70.622)	303±1.3** (55.3)	257±1.1** (58.43)	510±2.2* (58.55)	366±1.6** (61.709)
8 th day	949.99±4.2 (47.42)	971±9.7 (40.96)	262±1.1 (90.236)	345±1.5** (68.854)	240±1.0** (71.94)	504±2.2* (72.11)	121±0.54** (77.276)
10 th day	868.71±3.8 (55.48)	895±8.9 (52.96)	170±0.75 (94.946)	458±2.0** (80.83)	220±0.98* (82.82)	468±2.0* (83.03)	145±0.64** (87.364)
12 th day	949.99±4.2 (66.11)	359±3.5 (67.126)	58±0.25 (98.368)	246±1.0** (88.104)	157±0.70* (90.93)	400±1.7* (91.30)	118±0.52** (93.245)
14 th day	780.91±3.4 (77.14)	534±5.3 (74.648)	62±0.27 (99.548)	220±0.98** (94.56)	83±0.37** (96.41)	155±0.69** (95.72)	51±0.22** (97.84)
16 th day	477.94±2.1 (88.13)	486±4.8 (82.356)					

Day 0	Day 4	Day 8	Day12	Day 16	
					Base control
					Control
					Standard
					5% SA
					10% SA
					5% MO
					10 % MO

Figure 1: Photographical representation of effect of SA and MO on wound contraction in excision wound on different post excision day

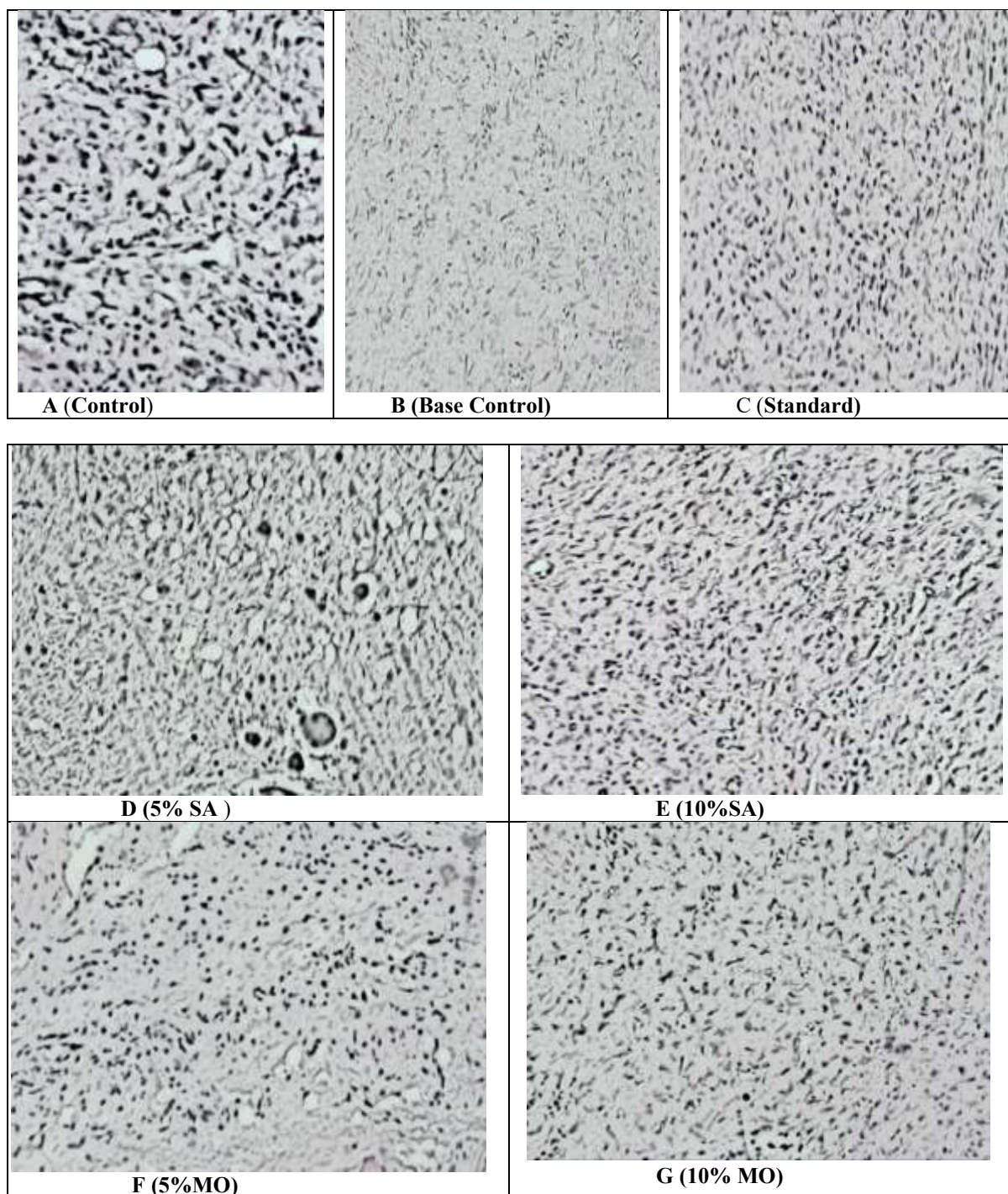


Figure 2: Section of the granulation tissues in the excision wound model A) stained with hematoxylin and eosin control group. B) Rat treated with simple ointment. Fibroblasts and neovascularization exhibit good collagen deposition while poor collagen is seen. C) 5% standard ointment was applied to the Rat. demonstrating healthy collagen deposition D) Rat given a 5%SA ointment treatment. displaying a considerable amount of collagen buildup E) 10% SA was applied to the rat. F) Rat given 5% MO treatment. G) A rat given 10% MO displaying increased collagen deposition and improved healing

DISCUSSION

The present study proved that the 10% ointment preparation of methanolic leaf extract of *Melissa officinalis* (MO) possessed significantly higher wound healing activity than *Streblus asper* (SA). This was reflected through a higher rate of wound contraction and reduced time taken for complete epithelization in MO-treated groups. Histological examination also validated these results, showing increased collagen fiber content and macrophage accumulation within the granulation tissues of the MO 10% ointment treated group (Figure 1, panels A–F). These findings indicate a more aggressive tissue regeneration process and enhanced inflammatory response modulation by the MO extract.

Another sign of improved wound healing in the SA-treated group was the raised tensile (breaking) strength of the newly formed tissue. This suggests improved maturation of collagen and perhaps enhanced cross-linkage among

collagen fibers, parameters that are of particular importance in assessing the mechanical stability and quality of healed skin.

Phytochemical assessment of SA and MO methanolic leaf extracts ensured the presence of important bioactive constituents including carbohydrates, glycosides, sterols, proteins, and flavonoids. There were, however, significant differences in their phytoconstituent profiles. MO extract had appreciable amounts of phenolic compounds (e.g., caffeic acid, chlorogenic acid, and rosmarinic acid), flavonoids (e.g., quercetin, luteolin, and kaempferol), and volatile compounds (e.g., citral and citronellal). Conversely, MO extract was richest in triterpenoids particularly ursolic acid (UA) as well as flavonoids, phytosterols, and cardiac glycosides⁵.

Ursolic acid which is a pentacyclic triterpenoid, is one of the principal bioactive compounds found in both MO and SA. UA has been widely researched for its pharmacological activities, including anti-inflammatory, anti-tumor, antioxidant, and cardioprotective properties. It has also shown promise in enhancing muscle regeneration and preventing muscle atrophy in in vivo models. Topically, UA is applied in dermatological formulations in various countries, including Japan, for the prevention and treatment of skin conditions like photoaging and skin cancer.^{18,19, 20, 21, 22}

It can behave like growth factors by initiating the activity of several cells engaged in wound healing, such as endothelial cells, fibroblasts, and myoblasts. The occurrence of such terpenoid components is likely to stimulate the synthesis of platelet-derived growth factors, which further support tissue repair mechanisms.^{23,24,25}

The superior activity of the 10% formulations may be attributed to a higher concentration of bioactive compounds such as **phenolics, flavonoids, terpenoids, and fatty acids**, which are known to promote collagen formation and epithelial regeneration.

Melissa officinalis exhibited greater wound healing efficacy compared to Streblus asper, which may be due to the presence of potent antioxidant compounds such as **rosmarinic acid, citral, and geraniol**, as identified by GC–MS analysis. These compounds help reduce oxidative stress at the wound site, thereby accelerating tissue repair. The reduction in epithelialization period suggests faster resurfacing of the wound, which is crucial for preventing microbial invasion. Antioxidant activity plays a key role in wound healing by neutralizing free radicals that otherwise delay the healing process. The correlation between antioxidant activity and wound healing observed in this study supports the traditional use of these plants in wound management.

Overall, the findings indicate that both plant extracts possess promising wound healing potential, with the **10% Melissa officinalis ointment showing the most significant effect**, closely comparable to the standard drug.

CONCLUSION

Scientific evaluation confirmed the antioxidant and Wound healing activity of methanolic leaves extract of MO and SA. GC–MS analysis of the extracts revealed a high concentration of bioactive phytochemicals. Both extracts exhibited pronounced antioxidant efficacy, demonstrated by significant free radical scavenging activity in DPPH assays. Collectively, these results indicate that Melissa officinalis leaf extract exhibited higher antioxidant activity compared to Streblus asper, which correlates with its higher content of phenolic and terpenoid compounds identified by GC–MS.

The effectiveness of its methanolic extracts highlights their potential for further preclinical and clinical investigation. Given its natural origin, this plant represents a promising, cost-effective source for developing herbal therapeutic agents with reduced risk of adverse effects. In conclusion, the research affirms that ointments formulated from 5% and 10% methanolic leaf extracts of Streblus asper (SA) and Melissa officinalis (MO) show increased wound healing activity than a plain ointment base (employed as the negative control). Between the two, the 10% MO formulation showed improved healing effects such as speeded-up wound contraction, shortened epithelialization time, and stronger tissue. The rich existence of phenolic and other terpenoids in MO is purported to be the key to its strong wound healing activity. The above findings constitute pharmacological rationale for the traditional application of SA and MO leaves in wound care and indicate that MO, specifically, is a potential candidate as a natural product source for the creation of new wound healing agents. Future studies focusing on the isolation and characterization of specific active compounds, their mechanisms of action, and potential for clinical application would be instrumental in further validating these findings.

Acknowledgement

The author thanks Maharishi School of Pharmaceutical Sciences, Maharishi University of Information Technology, IIM Road, Lucknow 226013, Uttar Pradesh, India Hygia Institute of Pharmaceutical Education and Research Faizullahganj, PrabhanNagar, Ghaila Road, Lucknow-226020 Uttar Pradesh, India for providing the infrastructure and facilities to do this research.

REFERENCE

1. Blanks, T. S., Brown, B., Cosgrave, J., Woody, V., Bentley, S., & Sullivan, N. (1998). The body shop book of well-being: Mind, body and soul (pp. 173–192). Ebury Press.
2. Patil, M. B., & Sunil, J. (2008). Antimicrobial and wound healing activities of leaves of Alternanthera sessilis Linn. International Journal of Green Pharmacy, 2, 141–144.

3. Kumar, R., Katoch, S. S., & Sharma, S. (2006). β -Adrenoceptor agonist treatment reverses denervation atrophy with augmentation of collagen proliferation in denervated mice gastrocnemius muscle. *Indian Journal of Experimental Biology*, 44, 371–376.
4. Lawrence, W. T., & Diegelmann, R. F. (1994). Growth factors in wound healing. *Clinical Dermatology*, 12, 157–169.
5. Falanga, V. (2004). The chronic wound: Impaired wound healing and solutions in the context of wound bed preparation. *Blood Cells, Molecules and Diseases*, 32, 88–94.
6. World Health Organization. (2004). WHO monographs on selected medicinal plants (Vol. 2: *Folium Melissae*). WHO Press.
7. Lin, J.-T., Chen, Y.-C., Lee, Y.-C., Hou, C.-W. R., Chen, F.-L., & Yang, D.-J. (2012). Antioxidant, antiproliferative and cyclooxygenase-2 inhibitory activities of ethanolic extracts from lemon balm (*Melissa officinalis* L.) leaves. *LWT – Food Science and Technology*, 49, 1–7. <https://doi.org/10.1016/j.lwt.2012.04.009>
8. Nascimento, G. G. F., Locatelli, J., Freitas, P. C., & Silva, G. L. (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Brazilian Journal of Microbiology*, 31, 247–256. <https://doi.org/10.1590/S1517-83822000000400003>
9. Venter, H., Henningsen, M. L., & Begg, S. L. (2017). Antimicrobial resistance in healthcare, agriculture and the environment: The biochemistry behind the headlines. *Essays in Biochemistry*, 61, 1–10.
10. Tundis, R., Loizzo, M. R., & Menichini, F. (2010). Natural products as α -amylase and α -glucosidase inhibitors and their hypoglycaemic potential in the treatment of diabetes: An update. *Mini Reviews in Medicinal Chemistry*, 10, 315–331.
11. brahim, N. M., Mat, I., Lim, V., & Ahmad, R. (2013). Antioxidant activity and phenolic content of *Streblus asper* leaves from various drying methods. *Antioxidants*, 2(3), 156–166.
12. Kumar, R. S., Kar, B., Dolai, N., Bala, A., & Halder, P. K. (2012). Evaluation of antihyperglycemic and antioxidant properties of *Streblus asper* Lour against streptozotocin-induced diabetes in rats. *Asian Pacific Journal of Tropical Disease*, 2(2), 139–143.
13. Sripanidkulchai, B., Junlatat, J., Wara-aswapati, N., & Hormdee, D. (2009). Anti-inflammatory effect of *Streblus asper* leaf extract in rats and its modulation on inflammation-associated gene expression in RAW 264.7 macrophage cells. *Journal of Ethnopharmacology*, 124(3), 566–570.
14. Shah, C. S., & Quadry, J. S. (1995). A textbook of pharmacognosy (11th ed.). Shah Prakashan.
15. Tekleyes, B., Huluka, S. A., Wondu, K., & Wondmkun, Y. T. (2021). Wound healing activity of 80% methanol leaf extract of *Zehneria scabra* (L.f.) Sond (Cucurbitaceae) in mice. *Journal of Experimental Pharmacology*, 13, 537–544.
16. Nayak, B. S. (2006). Wound healing activity of plants. *International Journal of Lower Extremity Wounds*, 5, 20.
17. Nayak, B. S., Nalabothu, P., Sandiford, S., Bhogadi, V., & Adogvo, A. (2006). Evaluation of wound healing activity of plants. *BMC Complementary and Alternative Medicine*, 6, 12.
18. Ofelia, C., Florin, B., Rita, A., & Iuliana, P. (2011). Syntheses of new cyclodextrin complexes with oleanolic and ursolic acids. *Journal of Agroalimentary Processes and Technologies*, 17, 405–409.
19. Liobikas, J., Majiene, D., Trumbeckaite, S., Kursvietiene, L., Masteikova, R., Kopustinskiene, D. M., Savickas, A., & Bernatoniene, J. (2011). Uncoupling and antioxidant effects of ursolic acid in isolated rat heart mitochondria. *Journal of Natural Products*, 74, 1640–1644.
20. Kunkel, S. D., Suneja, M., Ebert, S. M., Bongers, K. S., Fox, D. K., Malmberg, S. E., Alipour, F., & Shields, R. K. (2011). mRNA expression signatures of human skeletal muscle atrophy identify a natural compound that increases muscle mass. *Cell Metabolism*, 13, 627–638.
21. Ma, C. M., Cai, S. Q., Cui, J. R., Wang, R. Q., Tu, P. F., Hattori, M., & Daneshtalab, M. (2005). The cytotoxic activity of ursolic acid derivatives. *European Journal of Medicinal Chemistry*, 40, 582–589.
22. Ishida, M., Okubo, T., Koshimizu, K., Daito, H., Tokuda, H., Kin, T., Yamamoto, T., & Yamazaki, N. (1990). Topical preparations containing ursolic acid and/or oleanolic acid for prevention of skin cancer. *Chemical Abstracts*, 113, 12173.
23. Agrawal, S. S., & Paridhavi, M. (2007). Herbal drug technology (p. 323). Universities Press.
24. Somava, L. I., Shode, F. O., Ramnanan, P., & Nadar, A. (2003). Pharmacological evaluation of medicinal plants. *Journal of Ethnopharmacology*, 84, 299.
25. Harish, B. G., Krishna, V., Kumar, S. H., Ahmed, K. B., Sharath, R., & Swami, K. H. (2008). Antioxidant and antimicrobial activity of medicinal plants. *Phytomedicine*, 15, 763.