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Research Article

GC-MS Analysis and Wound Healing Potential of *Ficus racemosa* L. Gum in Wistar Albino Rats

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Abstract

Ficus racemosa is Ficus species plant which is popularly known as Country Fig, Udumbara and Gular. Traditionally it is use in various ailments such as ulcers, wound healing, diarrhea, stomachache and skin diseases. Skin wounds represent a major healthcare problem owing to an increasing number of trauma and pathophysiological conditions. Wound healing is a complex, well-orchestrated and regulated process consisting of series of events. Currently, available treatments are limited due to side effects and cost effectiveness. In line with that, we attempted to explore a natural source to study its potential towards the wound healing process. To the best of our knowledge, till date no documented information and published literature available reporting phytochemical profile using GC-MS (Gas chromatography and mass spectrometry) technique as well as animal studies related to healing properties of gum of *F. racemosa* plant. This work is the first report about the GC-MS analysis and healing potential of *F. racemosa* gum (FRG). GC-MS analysis of fine powder of FRG was performed by standard protocol using Perkin Elmer-Autosystem XL GC with TurboMass software which is, interfaced to MS Perkin Elmer TurboMass. GC-MS analysis identified three phytoconstituents such as, pentadecanoic acid (7.59%), hexadecyne (90.38%) and 1-undecene 9-methyl (2.04%). Then, its potential in wound healing on surgically wounded wistar rats was assessed by conducting two trials of 14 days and 24 days. During trials the wounds were visually observed, photographically documented and the wound area was measured. Results revealed that groups treated with *F. racemosa* gum (3%FRG and 1%FRG) showed considerable and progressive reduction in wound area (mm2) as well as significant percentage wound contraction and faster complete closure of wounds compare to control group. This study suggested that the gum of *F. racemosa* might be a potential therapeutic agent for skin wound healing, supporting its traditional medicinal uses.

Keywords: *Ficus Racemosa;* Gum; GS-MS; Phytochemical; Wound Healing

Introduction

Ficus racemosa belongs to Moraceae family which is found in various countries, including Australia, Malaysia, South-East Asia, Sri Lanka, Pakistan, China, New South Wales and the Indian subcontinent. According to ancient Ayurvedic, Siddha, Unani and Homeopathic traditions, *various parts of F. racemosa plant possess* various medicinal activities like as, antidiuretic, antitussive, antiulcer or gastro-protective, anti-oxidant, anthelmintic, antibacterial, antipyretic, anticholinesterase, antifilarial, wound healing, antidiarrheal, anti-inflammatory, antiulcer, analgesic, hepatoprotective and potential anticancer activities [1,2,7]. Different parts of *F. racemosa* plant contains various phytoconstituents that help in curing a variety of ailments such as diabetes, diarrhea ulcers, stomachache, piles, skin diseases, dysentery, as a carminative and in wound healing [6]. The leaves contain triterpenoids named gluanol acetate and racemosic acid, tannins, kaempferol, rutin, arabinose, bergapten, psoralenes, flavonoids, ficusin, coumarin, phenolic glycosides and saponins, bergaptol, lanosterol, β-Sitosterol etc. [3-5]. Fruits are found to contain sterols, triterpenoids, β-sitosterol, gluanol acetate, hentriacontane, tiglic acid of taraxasterol, lupeol acetate, gallic acid, ellagic acid and α-amyrin acetate, tiglic acid, taraxasterol etc. [5-7] Stem bark contains steroids, alkaloids, tannins named ellagic acid,

gluanol acetate, ceryl behenate, lupeol acetate, α-amyrin acetate, lupeol, friedelin, quercetin, bergenin, racemosic acid, β-amyrin and coumarin [8,9]. The roots of the plant contain euphorbol and its hexacosanoate, taraxerone, tinyatoxin, cycloartenol, ingenol and its triacetate, taraxerone etc. [8,10]. To the best of our knowledge very few research work has been done on pharmacological activity of different parts (leaves, fruits, stem bark etc.,) of *F. racemosa* tree. However, as per our search, we couldn't be able to find out a single animal study demonstrating the phytochemical screening and wound healing properties of *F. racemosa* gum. Based on the above discussed fact, the present study was planned to analysis a important phytochemicals in *F. racemosa* gum and evaluation of healing potential of gum using rat model.

A wound is defined as a disruption in the integrity of the skin caused by physical, thermal, chemical or microbial damage [11]. Wound healing is a process which ensures the restoration of skin integrity. It is a highly dynamic process and involves complex interactions of extracellular matrix molecules, soluble mediators, various resident cells and infiltrating leukocyte subtypes. Sometime wounds create a serious financial and medical burden, as well as considerable morbidity and death rates [12]. It is approximated that 1 to 2% of the population in developed countries suffers from a chronic wound throughout their lifespan [13]. The global impact of chronic wounds is derogatory with a prevalence rate of 6% [14]. In livestock, wounds are often neglected resulting into myiasis and huge economic losses. [15]. There is no satisfactory therapy available that fulfils all of the characteristics of a potential healing drug, such as potency, safety, innocuous, cost effectiveness and practicability. Therefore, we attempted to use a natural source to study its potential towards the wound healing process.

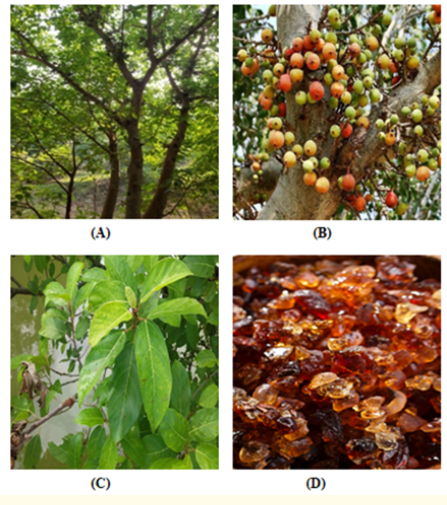


Figure 1: A) *F.racemosa* L. plant, B) *F.racemosa* fruits, (C) *F. racemosa* leaves, (D) *F. racemosa* gum.[16,17].

Material and Methods

Plant identification and authentification

The leaves and fruits of *F. racemosa* plant was collected from the campus of Indian Veterinary Research Institute (IVRI), Izatnagar, Bareilly, Uttar pradesh, while, gum was purchased from the local market of Bareilly. Herbariums of plant materials were given to Department of Botany, Banaras Hindu University (BHU), U.P., for identification and taxonomic authentification. The test report from Department of Botany, Banaras Hindu University (BHU), U.P. conformed the taxonomic authentification of plant material sample. The voucher specimen No. is *Ficus racemosa* L (Mora. 2022/01).

GC-MS analysis

- **Sample extraction**: A 500 mg of sample was dissolved in 9 ml Dichloromethane (DCM). The sample was extracted in a N_2 evaporator until it was concentrated to 1/4th volume of its initial volume. Obtained extract was evaporated to dryness and stored at 4°C in an airtight container for further use [18].

- **GC-MS protocol:** GC-MS analysis of this extract was performed using a Perkin Elmer- Autosystem XL GC with Turbomass and Gas chromatograph interfaced to a Mass Spectrometer Perkin Elmer Turbomass equipped with a Elite-5 ms, fused silica capillary column (30mm X0.25mm 1D X 0.5 µm, composed of 100% Dimethyl poly siloxane). For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1ml/min and an injection volume of 4µl was employed; Injector temperature 260^0 C; Ion-source temperature 220^0 C. The oven temperature was programmed from 75^0C (isothermal for 5 min), with an increase of 10^0 C/min, to 200^0 C, then 5^0 C/min to 280^0 C, ending with a 15 min isothermal at 280^0 C. Mass spectra were taken at 70 eV; a scan interval of 0.5seconds and fragments from 20 to 620 Da [19].

- **Interpretation of GC-MS results:** The relative % amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatograms was a Turbomass. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The Name, Molecular weight and structure of the components of the test materials were ascertained [20].

Experimental rats

Healthy adult male Wistar rats (150-170 g) were procured from Laboratory Animal Resource Section, ICAR-Indian Veterinary Research Institute (IVRI), Izatnagar (U.P.). The animals were housed in polypropylene cages with free access to standard feed and water. A balanced feed from Feed Technology Unit, IVRI was fed to the rats @ 15g/rat, twice daily throughout the study period. Before commencement of the experiment, all rats were kept in the laboratory condition for a minimum of 7 days for acclimatization [21]. The experimental protocols involved in this study were approved by the Institutional Animal Ethics Committee, Indian Veterinary Research Institute, Izatnagar, India as per permission No. IAEC/07.07.2020/S2.

Wound model

The rats were anesthetized by an intra-peritoneal injection of ketamine (50 mg/kg) and xylazine (5mg/kg) combination. A ~ 2x2 cm^2 (400 mm^2) open excision wound was created on the back (dorsal thoracic region) of the rats to the depth including panniculus carnosus. Animals, after recovery from anaesthesia, were individually housed in properly disinfected cages [21].

Herbal preparation and application

1% and 3% concentrations of *F. racemosa* gum powder and vehicle sesame oil (Cura, India) were used in herbal preparation for topical application. These concentrations of *F. racemosa* gum and vehicle were selected based on pilot study conducted in our laboratory. The herbal preparations were applied twice a day topically for 14 days in Trial I and for 24 days in trial II.

Experimental design

18 male rats were randomly divided into three groups of six animals each. Details of the experimental design of trial I and trial IIis given in table 1 and 2.

Groups	No. of animals	*F. racemosa* gum preparations	Collection of tissue
I	6	Vehicle control (Sesame oil)	Day 14
II	6	FRG (1% w/v)	Day 14
III	6	FRG (3% w/v)	Day 14

Table 1: Experimental protocol of Trial I (14 days) study.

Groups	No. of animals	*F. racemosa* gum preparations	Collection of tissue
I	6	Vehicle control (Sesame oil)	No collection of tissue. All animals kept upto complete wound closure
II	6	FRG (1% w/v)	
III	6	FRG (3% w/v)	

Table 2: Experimental protocol of Trial II (Complete wound closure) study.

Photographic evaluation and wound contraction measurements

In trial I, wounds were photographed and measured on days 0, 5, 9, 11 and 14 while in trial II on days 0, 5, 9, 11, 14, 17, 18, 19, 20, 21 and 24 post–wounding to assess the quality of wound healing. Wound area (mm^2) was measured by image analysis software ImageJ v1.53a [22]. The percent wound contraction was calculated by Wilson's formula as follows:

$$\%\ wound\ contraction = \frac{Day\ 0'wound\ area - wound\ area\ on\ a\ particular\ day}{'Day\ 0'woundarea} \times 100$$

Statistical analysis

Results are expressed as mean ± S.E.M, and the statistical significance between the treatment and control values was analysed by applying two-way analysis of variance followed by Bonferroni post tests using GraphPad Prism V.5. Software program (San Diego, CA, USA). A value of $p < 0.05$ was considered statistically significant when compared with control group. Values bearing superscripts not in common differ significantly [23].

Results

Phytochemical screening of *F. racemosa* gum by GC-MS

A total of 3 peaks were isolated and 3 phytoconstituents were identified by GC–MS analysis of FRG (Table 3 and Figure 2). Mass spectra of phytoconstituents are presented in figure 3. The phytoconstituents detected by GC-MS belong to fatty acyls, acetalides and unsaturated hydrocarbons (Table 4). GC-MS analysis of FRG indicated the presence of the pentadecanoic acid (7.59%), 1-hexadecyne (90.38%), 1-undecene, 9-methyl- (2.04%). These compounds exhibit antibacterial, antifungal and healing properties.

No.	Name of phytochemical	Retention Time	Area	Peak area%	Height	Molecular weight	Molecular formula
1	Pentadecanoic acid	19.11	1,401,652.9	7.59	5,352,207	242	C15H30O2
2	1-Hexadecyne	20.55	16,700,161.0	90.38	21,177,550	222	C6H30
3	1-Undecene, 9-Methyl-	25.40	376,074.3	2.04	2,607,757	168	C12H24

Table 3: Proposed retention time, area, peak area%, Molecular weight (MW) and Molecular formula of phytochemicals identified in GC-MS analysis of *F. racemosa* gum.

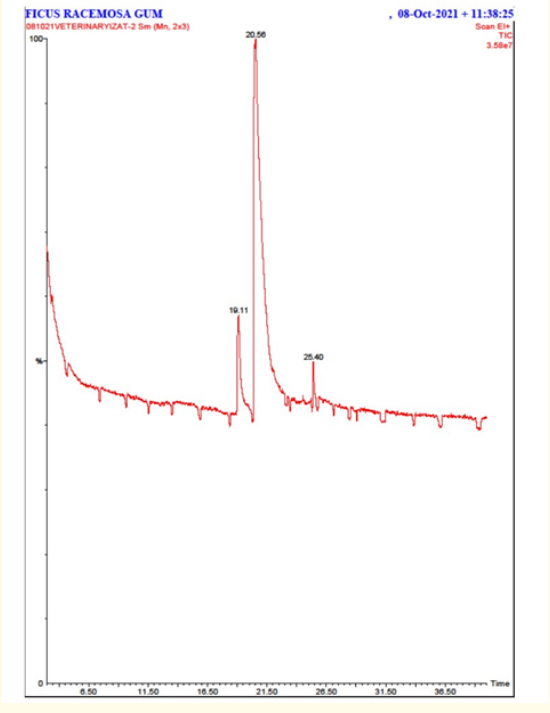
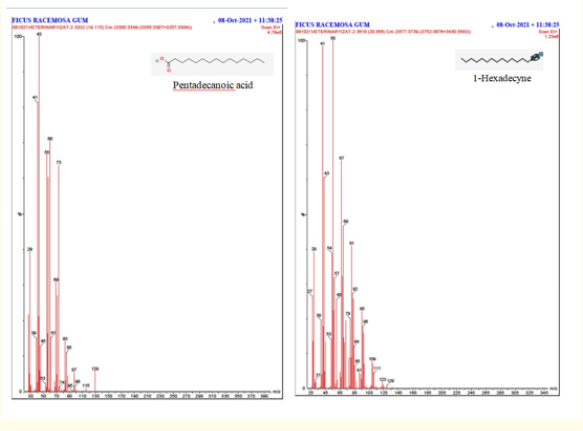


Figure 2: Gas chromatogram of identified phytochemicals from *F. racemosa* gum.



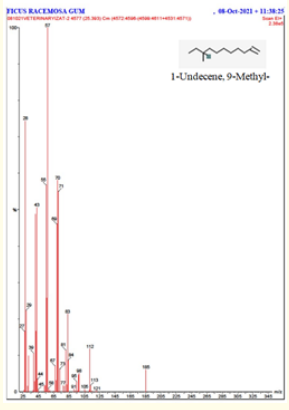


Figure 3: Mass spectra and chemical structures of identified phytochemicals (Pentadecanoic acid, 1-hexadecyne,1-undecene, 9-methyl-) in *F. racemosa* gum.

No.	Name of phytochemical	Nature of Phytochemical	Pharmacological Activity	CAS No.
1	Pentadecanoic acid	Fatty Acyls	Antibacterial, Antifungal [24]	1002-84-2
2	1-Hexadecyne	Acetalides	Antibacterial, Skin healing, [25]	629-74-3
3	1-Undecene, 9-Methyl-	Unsaturated hydrocarbons	Not Reported	74630-41-4

Table 4: Nature and pharmacological activities of major phytochemicals identified in GC-MS of FRG.

Effect of topical application of FRG on gross examination of wounds.

Trial I

Representative wound images, presented in figure 4 revealed that the wound size was progressively and considerably reduced in time dependent manner in all the treatment groups compared to control group. Moreover, among treatment groups, the reduction in wound size was higher in 3% FRG-treated group compare to 1% FRG-treated group.

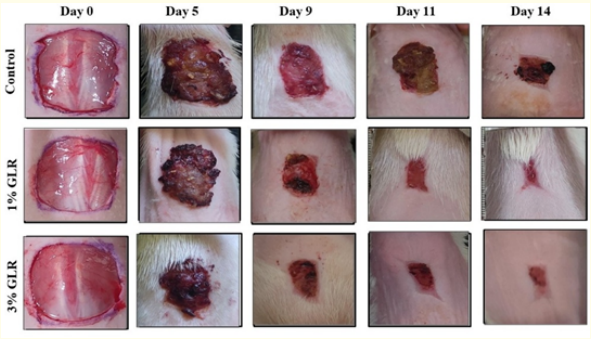


Figure 4: Representative gross images of wounds of control and *F. racemosa*-treated groups on days 0, 3, 7, 11 and 14 post-wounding.

Trial II

Photographs of wounds on different days upto complete wound closure (CWC) are presented in figure 5 demonstrated that the wound size was progressively reduced in time dependent manner in the treatment groups compared to control group. 3% FRG-treated showed faster decrease in wound size which is followed by 1% FRG-treated group while, wounds of control group indicated slow rate of healing process. Treatment groups showed regrowth of hair in wounded area.

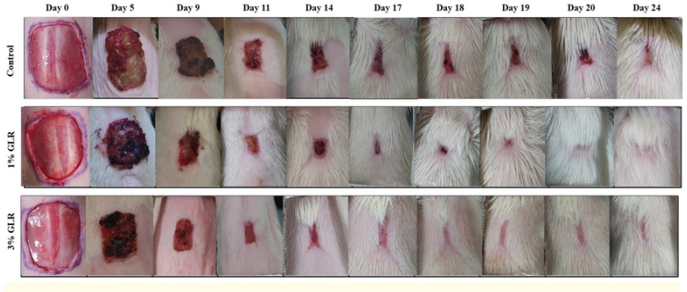


Figure 5: Representative gross images of wounds of control and *F. racemosa*-treated groupsupto complete wound closure.

Effect of topical application of FRG on wound closure (absolute area mm_2)

Trial I

The absolute measurements of wounds closure (area mm^2) are tabulated in Table 5 indicated that, From 5^{th} to 14^{th} day all the treatment groups are shown significant wound closure compare to control group. Wound closure was fastest in 3% FRG-treated group.

Groups	Control	1% FRG	3% FRG
Day 0	412.45 ± 18.56	403.26 ± 14.21	408.07 ± 20.23
Day 5	315.56 ± 12.23	269.42 ± 11.56	254.26 ± 17.64
Day 9	160.12 ± 8.35	137.08 ± 9.14	109.38 ± 6.12
Day 11	89.56 ± 5.12	72.14 ± 7.21	46.76 ± 3.18
Day 14	61.21 ± 3.59	34.21 ± 2.84	21.42 ± 4.20

Table 5: Effect of topical application of FRG on wound closure (absolute area mm^2) in trial I study.

Trial II

The absolute measurements of wounds closure (area mm^2) tabulated in Table 6 revealed that, 3% FRG-treated group showed fastest wound closure which was followed by 1% FRG-treated group while control group showed slowest wound closure among all groups. Among all groups, wound closure takes 17, 19 and 24 days for 3% FRG, 1% FRG, and control groups, respectively.

Groups	Control	1% FRG	3% FRG
Day 0	410.12 ± 16.08	417.26 ± 10.26	418.16 ± 12.48
Day 5	308.74 ± 11.23	281.96 ± 9.42	268.08 ± 8.96
Day 9	168.82 ± 9.46	127.36 ± 6.28	115.34 ± 6.24
Day 11	102.00 ± 8.84	61.90 ± 7.26	39.12 ± 3.42
Day 14	70.21 ± 6.52	22.62 ± 5.30	12.21 ± 1.58
Day 17	56.12 ± 6.48	10.43 ± 2.16	0.00
Day 18	34.31 ± 4.32	4.28 ± 1.18	CWC
Day 19	22.18 ± 5.56	0.00	CWC
Day 20	12.41 ± 2.68	CWC	CWC
Day 24	5.24 ± 2.42	CWC	CWC

Table 6: Effect of topical application of FRG on wound closure (absolute area mm^2) in trial II study.

Effect of topical application of FRG on wound contraction (%)

Trial I

Topical application of *F. racemosa* gum decrease the size of wound by significantly increasing percent wound contraction (Table 7 and Figure 6) as compared to control group. Wound contraction was highest in 3% FRG- treated group. On Day 11, 1% FRG-treated group showed no significant increase in wound contraction compare to control group. On Day 14, all the treatment groups showed significant increase in wound contraction compared to control group.

Groups	Control	1% FRG	3% FRG
Day 5	23.53 ± 3.42[a]	33.25 ± 4.28[b]	37.74 ± 5.20[c]
Day 9	61.69 ± 5.21[a]	65.92 ± 4.10[b]	73.28 ± 3.36[c]
Day 11	78.26 ± 2.81[a]	82.13 ± 2.16[a]	88.72 ± 1.06[b]
Day 14	85.16 ± 1.42[a]	91.51 ± 1.02[b]	94.85 ± 1.16[c]

Table 7: Effect of topical application of FRG on wound contraction (%) in trial I study.

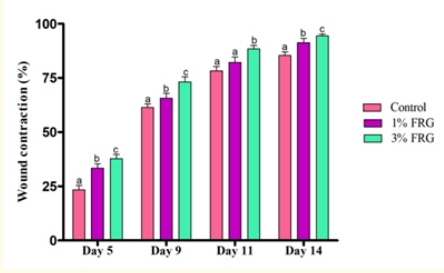


Figure 6: Effect of topical application of *F. racemosa* gum on wound contraction (%) on days 5,9, 11 and 14 post-wounding.

Trial II

In trail II, the wound contraction was significantly high in FRG-treated groups from day 5 onwards. The 3% FRG-treated group had the fastest wound contraction rate and it could completely cure wounds in 17 days, while the 1% FRG group may heal wounds in 19 days. The control group had the slowest rate of wound contraction, which takes 24 days to heal. (Table 8 and Figure7).

Groups	Control	1% FRG	3% FRG
Day 5	24.87 ± 5.21a	32.85 ± 4.68[b]	35.21 ± 3.16[c]
Day 9	59.02 ± 4.16a	69.47 ± 2.16[b]	72.48 ± 2.46[c]
Day 11	75.12 ± 4.20a	85.15 ± 3.78[b]	90.64 ± 2.28[c]
Day 14	82.87 ± 3.15a	94.5 ± 2.04[b]	96.83 ± 1.08[c]
Day 17	86.31 ± 3.04a	97.50 ± 2.34[b]	100.00 ± 0.00[c]
Day 18	91.63 ± 3.26a	98.99 ± 1.26[b]	CWC
Day 19	94.60 ± 2.11a	100.00 ± 0.00[b]	CWC
Day 20	97.04 ± 1.20	CWC	CWC
Day 24	98.73 ± 0.87	CWC	CWC

Table 8: Effect of topical application of FRG on wound contraction (%) in trial II study.

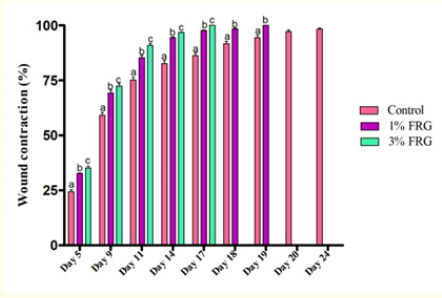


Figure 7: Effect of topical application of *F. racemosa* gum on wound contraction (%) upto complete wound closure.

Discussion

Medicinal plants are important source of synthetic and herbal drugs. Virtually all medicinal plants have bioactive principles which are responsible for most of the biological activities they exhibit. Knowledge of the phytochemicals of plants is desirable for the discovery of therapeutic agents as well as for revelation of new sources of economically valuable phytocompounds [26]. Hence, in our study we performed phytochemicals screening of *F. racemosa* gum by GC-MS technique. Results indicated the presence of the pentadecanoic acid (7.59%), 1-hexadecyne (90.38%), 1-undecene, 9-methyl- (2.04%). Reported possible pharmacological activities of these compounds are antibacterial, antifungal and healing properties [24,25]. Compounds' antimicrobial activities like antibacterial and antifungal contribute in the healing process by preventing microbial growth in wound area [27]. Therefore, we can correlate that the bioactive compounds identified in *F. racemosa* gum may be responsible for the ethnomedical or traditional use of this plant.

Cutaneous wound healing is a dynamic process that involves a series of overlapping phases, including, inflammation and granulation tissue formation which includes re-epithelialization and angiogenesis, and matrix remodeling. A fast closure of the skin defect is essential to prevent infection and further damage to the internal tissue [28]. The sensitive balance between the stimulating and inhibitory mediators of the wound repair process is crucial to achieve an early and fast wound contraction/closure following injury. Wound contraction is the movement of wound edges towards each other in a centripetal fashion [29]. Contraction of the wound begins soon after wounding and peaks at 2 weeks. Myofibroblasts are the predominant mediator of this contractile process because of their ability to extend and retract. During granulation tissue formation, fibroblasts are gradually modulated into myofibroblasts

[30]. In the present study also, we observed significant increase in per cent wound contraction, and moreover there was considerable reduction in wound size and wound area in *F. racemosa* gum-treated groups from day 5 to 14 compared to control group. *F. racemosa* gum promote wound healing by accelerating complete wound closure along with regrowth of hair in wounded area. Our study results are supported by few reported studies such as, in the excision wound healing model, the 5% w/w alcohol extract of *F. racemosa* and enriched diet showed an increase in percentage wound closure by promoting epithelialization and collagen synthesis [31]. On cutaneous wound healing. 5% w/w and 10% w/w formulation of methanolic extract of leaves of *Ficus religiosa* showed complete healing in 24.74 and 21.53 days respectively, as compared to untreated group which took 30.38 days [32]. Aqueous and ethanolic extract of root of *Ficus benghalensis* showed a marked increase in breaking strength and wound closure rate by increasing epithelialization and enhanced collagen synthesis. The ethanolic extract showed wound healing in 17.16 days whereas aqueous extract took 18.33 days, as compared to control group which took 21.50 days. [8,33] So, our findings suggests that there was marked acceleration of wound healing process after application on wound, which may be due to the stimulatory effect of *F. racemosa* gum preparations on complete wound closure, wound contraction and transformation of fibroblasts to myofibroblasts.

Conclusion

GC-MS analysis revealed that *F. racemosa* gum contains phytochemicals that are related to its traditional use. The topical application of sesame oil based *F. racemosa* gum preparation has healing potential in excision wound model may be because of a remarkable improvement in complete wound closure, percentage wound contraction and transformation of fibroblasts to myofibroblasts.

Future Prospects

Additional research is required to identify and isolate the essential phytochemicals that enhance wound healing using other modern analysis techniques. Other complementary animal studies and research, dealing with the optimization of the *F. racemosa* gum concentration to be used for skin healing in order to establish the herbal formulation.

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