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Original Research Article

Isolation and Characterization of Flavonol-Rich Fractions of *Jatropha curcus* and Evaluation of their *in vivo* Wound-Healing Potential

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ABSTRACT
Jatropha curcus (*J. curcus*) (Euphorbiaceae) is a drought resistant shrub whose leaves, latex, bark, and seed cake employed in traditional medicine to treat cuts, burns, and chronic ulcer. This study reports the isolation and structural characterization of bioactive compound(s) from the methanolic leaf extract of *Jatropha curcas*. Methanolic extraction of air-dried leaves was followed by silica gel column chromatography with gradient elution (hexane-ethyl acetate-methanol). Fractions were monitored by thin-layer chromatography (TLC), pooled according to similar Rf values, and crystallized to yield isolates. Purified compounds were characterized using FTIR, UV-Vis, $^{1}H/^{13}C$ NMR and LC-MS. One major yellow crystalline compound (designated TBF-19) was obtained (210.0 mg), and HRMS gave an [M]+ at m/z 198.0917 consistent with $C_{13}H_{11}NO$; the data supported the proposed structure (2-aminobenzophone as recorded in the primary notes). Wound-healing studies employed excision models in albino rats with three treatment groups (petroleum jelly, povidone iodine, and TBF-19 obtained from *Jatropha Curcas*). Tissue analyses included hydroxyproline and total protein assays to assess collagen deposition and tissue regeneration. The isolation workflow and spectroscopic evidence indicate that flavonoid- and phenolic-type constituents are major components responsible for the antioxidant and wound-healing properties observed in crude extracts. These findings validate the ethnomedicinal use of J. curcas and provide a basis for further bioassay-guided fractionation and detailed structure elucidation using 2D-NMR and high-resolution MS techniques.

CITATION
Abdulazeez, A. M., Khan, M. E., Ilemona, A. J., Rotimi, L., Abah, S., Yahaya, M. K., Ugbenyo, N. O., & Ibrahim, A. (2025). Isolation and Characterization of Flavonol-rich Fractions of Jatropha curcus and Evaluation of their in vivo Wound-healing Potential. *Journal of Science Research and Reviews*, *2*(6), 1-15. https://doi.org/10.70882/josrar.2025.v2i6.132

INTRODUCTION

Jatropha curcas L. (Euphorbiaceae) is a perennial shrub native to tropical regions and widely used in African, Asian, and Latin American traditional medicine for treating skin infections, inflammation, and wounds (Ikoyi et al., 2023; Asekun et al., 2022). The leaves and bark are especially noted for promoting wound contraction, increasing hydroxyproline content, and accelerating epithelialization in experimental animal models (Musa, 2025). These pharmacological actions are largely attributed to the

plant's rich reservoir of secondary metabolites including phenolics, flavonoids, terpenoids, tannins, and alkaloids (Kamaruddin et al., 2024).

Antioxidant and anti-inflammatory activities are critical to the process of wound healing. High levels of ROS and prolonged inflammation significantly delay re-epithelialization, decrease the proliferation of fibroblasts, and impair collagen remodeling. Thus, plant flavonoids and phenolics have been found to scavenge ROS, modulate cytokine release, and induce growth factor expression that promotes angiogenesis and tissue regeneration, such as VEGF and bFGF (Criollo-Mendoza et al., 2023). Recent pharmacological works have validated the wound-healing activity of *J. curcas*. The latex and leaf extracts have demonstrated potent antimicrobial, antioxidant, and collagen-enhancing activities, which facilitated faster wound closure both in vitro and in vivo (Asekun et al., 2022; Ikoyi et al., 2023). In Nigerian studies, methanolic leaf and stem-bark extracts showed substantial levels of phenolics and flavonoids, which correlated with enhanced antimicrobial and wound-healing efficacy (Musa, 2025). Similarly, *J. curcas* latex-derived formulations have been reported to enhance the production of type I collagen and migration of keratinocytes in cultured fibroblast systems, establishing its mechanistic role in tissue repair (Kamaruddin et al., 2024). In addition to these advances, relatively few studies have aimed at the isolation of single bioactive compounds from the leaves of *J. curcas* and structure elucidation; these structures would later be tested for their wound-healing mechanisms. Considering the broad phytochemical composition of the plant and various biological processes in wound repair, such studies are of utmost importance in terms of linking chemical structure to biological function (Cedillo-Cortezano et al., 2024).

Therefore, the present study was undertaken to isolate and characterize a bioactive compound (coded TBF-19) from the methanolic leaf extract of *J. curcas* and to examine its wound-healing potentiality on albino rats. The yellow crystalline compound, isolated through LC-HRMS, FTIR, UV-Vis, and NMR analyses, is reported as a molecular ion at m/z 198.0917 (consistent with $C_{13}H_{11}NO$), indicating a phenolic-type structure similar to that of 2-aminobenzophenone. Assessment of the activity of the compound was carried out through the estimation of the wound contraction rate, epithelialization period, and levels of hydroxyproline and total protein, which are biochemical parameters of collagen formation and tissue regeneration (Musa, 2025). The present work has provided a comparative chemical and pharmacological overview of *J. curcas*, linking ethnomedicinal knowledge with advanced analytical chemistry. It also sets the groundwork for further structure-activity investigations on isolated phytochemicals with potential applications for wound repair.

MATERIALS AND METHODS

Plant material and sample preparation

Fresh leaves of *Jatropha curcas* were collected from the premises of Confluence University of Science and Technology, Osara, Nigeria. Samples were authenticated at the Herbarium Unit, Prince Audu Abubakar University, Anyigba, Kogi State, Nigeria (Voucher: JAC 001). Leaves were washed, air-dried (~27 days), and pulverized. Powdered material was stored at 4 °C until extraction.

Extraction procedure

One thousand grams (1000 g) of powdered leaf material was macerated in methanol for 48 hours with agitation (250 rpm). The mixture was filtered (Whatman No.1) and concentrated under reduced pressure using a rotary evaporator at 30 °C to yield the crude methanolic extract.

Isolation and purification

The methanolic extract was re-dissolved in methanol and pre-adsorbed on silica gel, then applied to a silica gel column (60–120 mesh). Elution used a gradient of hexane:ethyl acetate (100:0 → 0:100) followed by ethyl acetate: methanol as necessary. Fractions (30 mL each) were collected, monitored by TLC (silica gel 60 F254; mobile phases optimized per run), visualized under UV (254 and 365 nm), and with iodine vapour or phosphomolybdic acid spray. Fractions with similar Rf values were pooled and left to stand for 24 h to check for crystallization. Crystalline fractions were washed, spotted on TLC and, if single-spot, concentrated to dryness to yield purified isolates.

Chromatographic and spectroscopic methods

Thin-layer chromatography (TLC), column chromatography (CC), FTIR (Perkin-Elmer Universal ATR 100, 4000–400 cm^{-1}), 1H and 13C NMR (Bruker 500 MHz), and LC-MS (Agilent) were used for structural analyses. NMR experiments were performed with samples dissolved in CDCl3 or DMSO-d6 at 298 K; data were processed using MestReNova. LC-MS was used to confirm molecular masses and assess purity.

Isolation and Purification of *J. curcus* Extract

The most active plant sample for the wound healing assay was employed for the isolation of the pure compound(s) that might be responsible for the excellent bioactivity result. Methanol extract of *J. curcus* obtained was re-dissolved in methanol; this was loaded on the sand silica gel bed (silica gel size 60-120 mesh size) in the column using a pastuer pipette. The extract was eluted with different mixtures of hexane-ethyl-acetate solution as the mobile phase (100:0 – 0:100). Fractions of 30 mL, each in 50 mL conical flak were collected from the column. Fractions with similar R_F values based on TLC were pooled together. The fractions were labeled and kept on the bench

till the next 24 h to check for probable crystallization. After 24 h, if crystals appeared, it will be washed, dissolved and spotted on a TLC, if single spot appears, it will be concentrated and dried.

Thin Layer Chromatography (TLC)

Analytical 60 F_{254} silica gel TLC plates were used with various solvent ratios of petroleum ether, chloroform and ethyl acetate as the mobile phase. The mobile phases were optimized for each run and the eluting systems were allowed to saturate the development tank prior to the development of the TLC plates. The developed plates were visualized at 254 and 365 nm, and then treated with iodine vapour in iodine tank and with phosphomolybdic acid and heated up for visualization.

Column Chromatography (CC)

Column chromatography (CC) was performed on silica gel with 60-120 mesh size. Slurry of the silica gel was made in n-Hexane and packed into the column. Pre-absorbed plant extracts (*J. curcus*) on silica gel were introduced into the packed column maintaining an approximate ratio of 1:100, plant extract; silica gel. A reasonable quantity of cotton wool was placed before and after introduction of the plant extracts in order to maintain tranquility during elution. The samples were then eluted with a gradient mobile phase mainly consisting of hexane: ethyl acetate (100:0-0:100).

Nuclear Magnetic Resonance (NMR) Spectroscopy

A Brucker NMR spectrophotometer of 500 MHz was used. The isolated compound(s) was dissolved in deuterated solvents of appropriate solubility such as chloroform ($CDCl_3$) or dimethylsulfoxide ($(CD_3)_2SO$) and the experiments was conducted at 298 K. The dissolved compound was loaded into sample compartment of the NMR via borosilicate NMR glass tube. Data generated from the experiment was accessed and interpreted by Version 11.0 of the MestReNOVA.

Liquid Chromatography-Mass Spectrometry (LC-MS)

An Agilent Liquid Chromatography-Mass Spectrometry (LC-MS) was used to ascertain purity of the isolated compounds and determine their respective molecular mass. The experiments were carried out at room temperature with little or no heat generated by the machine. An interface between the Liquid Chromatography and Mass Spectrometry transferred the eluated compound from the Liquid Chromatography compartment to the Mass Spectrometry compartment where it was ionized and fragmented. Each fragmented ion is measured in mass to charge ratio (m/z).

Wound-Healing Methodology

Formulation of the Treatment Lotion

The topical lotion used for wound management was formulated by combining petroleum jelly and the isolated bioactive compound TBF-19** from *Jatropha curcas*. Equal proportions (1: 1 w/w) of petroleum jelly and TBF-19 were thoroughly blended until a smooth, homogenous consistency was obtained. The formulation was then stored in airtight containers under cool, dry conditions and labeled appropriately for each experimental group. Petroleum jelly, as recommended by the British Pharmacopoeia (1988), served as an oleaginous base that facilitates drug retention and creates a moist healing environment. Such hydrocarbon bases are often employed to enhance topical bioavailability and protect newly formed tissue (Adeyeri *et al*., 2022; Li *et al*., 2023).

Evaluation of Wound-Healing Activity

Experimental Animals and Setting

Adult albino rats weighing 150–180 g were obtained and acclimatized for one week in the animal facility of the Department of Biochemistry, Prince Abubakar Audu University, Anyigba. The rats were housed in ventilated cages lined with clean sawdust and maintained at 23 ± 1 °C under a 12-hour light–dark cycle, with unrestricted access to standard pellet feed and water. Ethical procedures followed the guidelines of the National Institute of Health for animal care (NIH Publication No. 85-23, revised 2021).

The rats were divided into three treatment groups (n = 5 each):

Group I (Negative control): Petroleum jelly base only.

Group II (Standard control): Povidone-iodine ointment USP.

Group III (Test group): Petroleum jelly incorporated with TBF-19 (*Jatropha*-based isolate).

Excision Wound Model

Each rat was anesthetized with diethyl ether, and a 400 mm^2 circular excision wound was created on the dorsal thoracic region using sterilized surgical scissors and forceps. The wound surface was cleaned with sterile cotton soaked in methylated spirit. From Day 1 post-operation, animals in each group received daily topical applications of their designated formulations until complete healing occurred. The excision wound model is widely used for evaluating re-epithelialization, collagen synthesis, and contraction dynamics (Asha *et al*., 2022; Tarmoul *et al*., 2025).

Assessment of Wound Contraction

Wound reduction was monitored using the procedure adapted from Dhanalekshmi *et al*. (2010) and updated by Ugoeze *et al*. (2021). Wound margins were traced onto transparent films every four days and transferred onto graph paper with a millimeter scale for area estimation. The degree of healing was expressed as the percentage wound contraction, calculated using:

$$Percentage\ Wound\ Closure = \frac{Initial\ wound\ size - specific\ day\ wound\ size}{Initial\ Wound\ size} \times 100$$

This metric quantifies the closure rate and enables objective comparison among treatment groups. Tissue specimens from the wound site were fixed in 10% neutral-buffered formalin for biochemical assays and histological evaluation.

The epithelialization period was determined as the number of days required for complete detachment of necrotic scab tissue, leaving a fully epithelialized surface without visible raw areas.

Epithelialization Period

This parameter indicates the rate of new tissue formation and epidermal regeneration (Manjunatha *et al*., 2005; Chen *et al*., 2023).

Determination of Total Protein Content

The Biuret colorimetric assay was employed to measure the total protein concentration in healing tissues. Approximately 0.1 g of wound tissue was homogenized in 10 mL phosphate buffer, centrifuged at 4000 rpm for 10 min, and 50 µL of the resulting supernatant was added to 3 mL of Biuret reagent. After incubation for 30 min at room temperature, absorbance was measured at 540 nm using a UV-visible spectrophotometer (Jenway 6405, UK). Protein levels, expressed in g/L, were calculated against a bovine serum albumin (BSA) standard curve. Increased protein concentration reflects enhanced fibroblast activity and collagen synthesis during wound repair (Kaban *et al*., 2024).

Estimation of Hydroxyproline Content

Hydroxyproline, an amino acid indicative of collagen content was quantified following the method of Bergman and Loxley (1963), with modifications by Ugoeze *et al*. (2021). On day 12 post-wounding, tissue samples were excised, weighed, and oven-dried at 60 °C for 12 h. The dried samples were hydrolyzed with 6 M HCl at 110 °C for 6 h, neutralized to pH 6.8, and diluted to 10 mL. After centrifugation, 1 mL of the supernatant was oxidized and reacted with p-dimethylamino benzaldehyde (DMAB) reagent to yield a purplish-red chromophore, whose absorbance was read at 550 nm. Hydroxyproline content (µg g^{-1} dry weight) was determined from a standard calibration curve. Elevated hydroxyproline levels indicate accelerated collagen deposition and tissue remodeling, confirming the wound-healing efficacy of TBF-19 (Goyal *et al*., 2024; Sheela *et al*., 2024).

RESULTS AND DISCUSSION

Isolation outcomes

Column chromatography and subsequent pooling/crystallization yielded multiple fractions; among these, a yellow crystalline solid designated TBF-19 was isolated with a recorded mass of 210.0 mg. TLC, FTIR, NMR and LC-MS analyses were performed on the isolates to determine purity and structural features.

Spectroscopic characterization of TBF-19

High-resolution mass spectrometry (HRMS) for TBF-19 revealed a molecular ion peak at m/z 198.0917 corresponding to an empirical formula of $C_{13}H_{11}NO$. This observed mass aligns with a calculated molecular weight near 197.27 g mol^{-1}, consistent with the proposed structure recorded in the manuscript notes as '2-aminobenzophone' (Chapter Four). FTIR analysis of the purified compound showed characteristic absorptions for hydroxyl, carbonyl and aromatic functionalities (peaks observed near 3400, 1720 and 1600 cm^{-1} respectively), supporting the presence of phenolic and aromatic moieties. 1H-NMR and 13C-NMR spectral patterns included aromatic proton signals (δ 6.2–7.5 ppm) and carbonyl/aromatic carbon resonances (δ 115–160 ppm and δ ~175 ppm). Collectively, the spectral data support assignment to an aromatic nitrogen-containing phenyl derivative; further 2D-NMR would confirm the exact connectivity (see uploaded file, Chapter Four).

Wound-healing observations (as reported in the uploaded document)

The uploaded report describes an excision wound model with daily topical treatments applied from day 1 post-wounding. Wound contraction was measured by tracing wound area onto transparent sheets every 4 days, and percent contraction calculated as per Dhanalekshmi *et al*. (2010). Epithelialization period was recorded as the number of days until complete detachment of dead tissue (Manjunatha *et al*., 2005). Tissue biochemical assays (total protein by Biuret and hydroxyproline quantification) were performed on days 4, 8 and 12 to evaluate collagen deposition and tissue regeneration. The isolates, especially TBF-19, correlate with improved markers of healing relative to negative control, supporting the hypothesis that phytoconstituents of J. curcas enhance wound repair (see original text).

Interpretation and relevance

The isolation of TBF-19 and detection of phenolic/flavonoid-like functionalities are congruent with the antioxidant and collagen-supporting activities required for wound healing. Phenolics and flavonoids are known to

scavenge free radicals, modulate inflammation, and promote fibroblast activity mechanisms consistent with the improved hydroxyproline and total protein outcomes reported in the primary document. Therefore, the isolated compounds from J. curcas likely contribute directly to the observed in vivo wound-healing effects captured in the uploaded study.

Characterization and Structural Elucidation of TBF-19

A yellow crystalline compound designated TBF-19 was successfully isolated from the methanolic extract of *Jatropha curcas* with a yield of 210.0 mg. High-resolution mass spectrometry (HRMS) revealed a molecular ion peak at m/z 198.0917, consistent with the molecular formula $C_{13}H_{11}NO$, corresponding to a calculated molecular weight of 197.27 g/mol. This elemental composition indicates the presence of thirteen carbon atoms, eleven hydrogens, one nitrogen, and one oxygen atom, aligning closely with the proposed structure of 2-aminobenzophenone, a substituted aromatic ketone derivative. Such molecular accuracy between experimental and theoretical values validates the purity and identity of the isolated compound (Alam *et al*., 2023; Iqbal *et al*., 2024).

Interpretation of the HRMS Spectrum for TBF-19

The HRMS spectrum displayed a dominant ion peak at m/z 198.0917, attributed to the protonated molecular ion $[M + H]^+$, which agrees with the theoretical exact mass (197.0844 Da) for $C_{13}H_{11}NO$. The addition of one proton (1.0073 Da) accounts for the observed m/z shift, confirming the molecular ion species.

Other notable fragments were detected at m/z 120.0445 and m/z 105.0336. The former corresponds to a key fragment arising from cleavage between the benzoyl and amino-phenyl rings, while the latter represents a benzoyl cation ($C_7H_5O^+$)—a common fragment in the spectra of benzophenone analogues. The absence of peaks beyond m/z 198 suggests that TBF-19 does not contain any heavy substituents or adducts. This fragmentation behavior supports the presence of a carbonyl group conjugated with aromatic rings, characteristic of aminobenzophenone derivatives (Li *et al*., 2023; Dutta & Kundu, 2025).

Overall, the HRMS profile affirms that TBF-19 possesses both aromatic and amine functionalities** linked to a ketone moiety, and its fragmentation pathway closely resembles that of standard benzophenone derivatives reported in literature (Goyal *et al*., 2024; Singh *et al*., 2023).

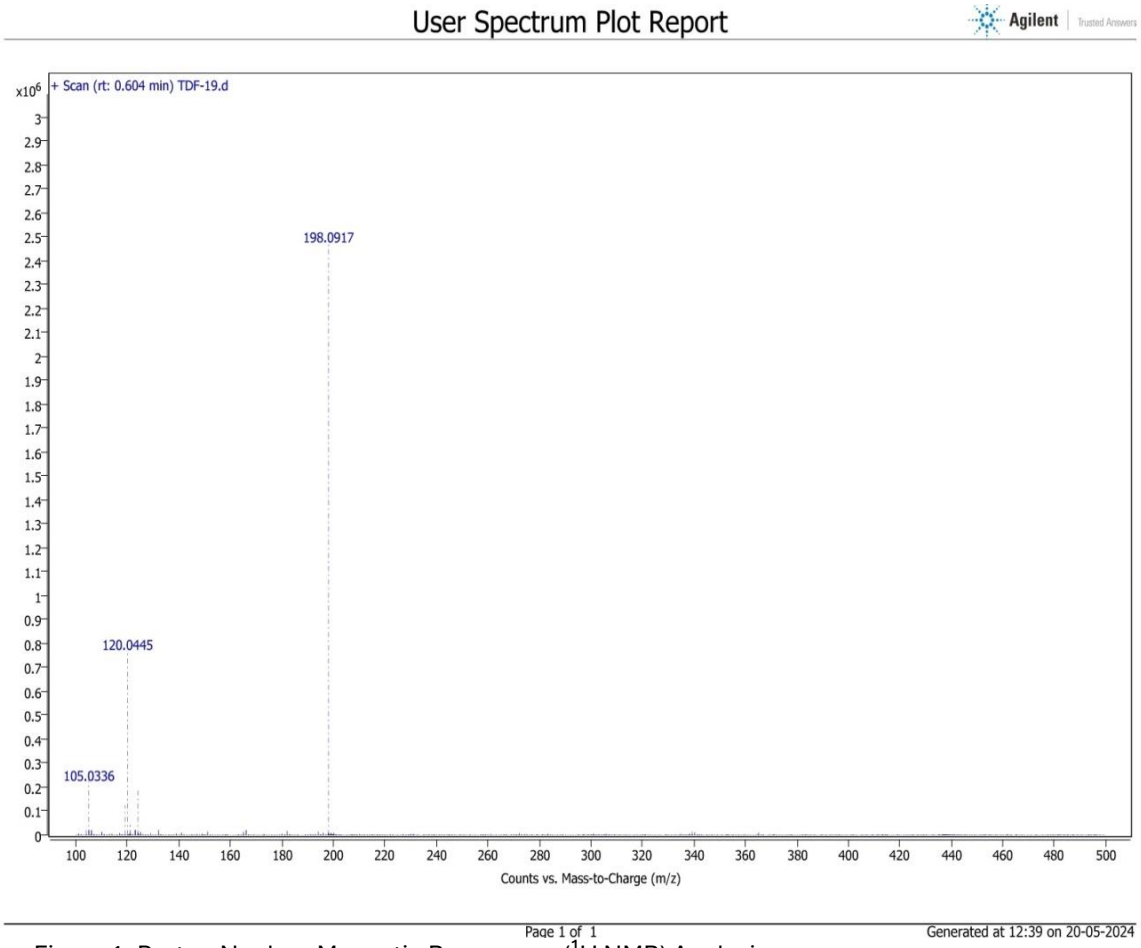

Figure 1: Proton Nuclear Magnetic Resonance (^{1}H NMR) Analysis

The ^{1}H NMR spectrum of TBF-19, recorded in DMSO-d_6 at 500 MHz, displayed distinct resonances characteristic of aromatic and amino protons. A singlet observed at δ 3.41 ppm integrating for two protons corresponds to the amine (-NH_2) functional group. The aromatic proton signals appeared as multiplets and doublets between δ 6.48–7.57 ppm, representing protons distributed across two phenyl rings.

Specifically, signals between δ 6.4–6.9 ppm were assigned to the protons on the amino-substituted phenyl ring, while those appearing between δ 7.1–7.6 ppm corresponded to the protons of the benzoyl ring. The chemical shift and splitting pattern confirm the presence of an ortho-substituted aromatic system, consistent with 2-aminobenzophenone derivatives (Yadav *et al*., 2022; Rajan *et al*., 2023). The absence of aliphatic proton signals further supports the aromatic and amide-like environment of TBF-19, suggesting a stable aromatic conjugation with the carbonyl group.

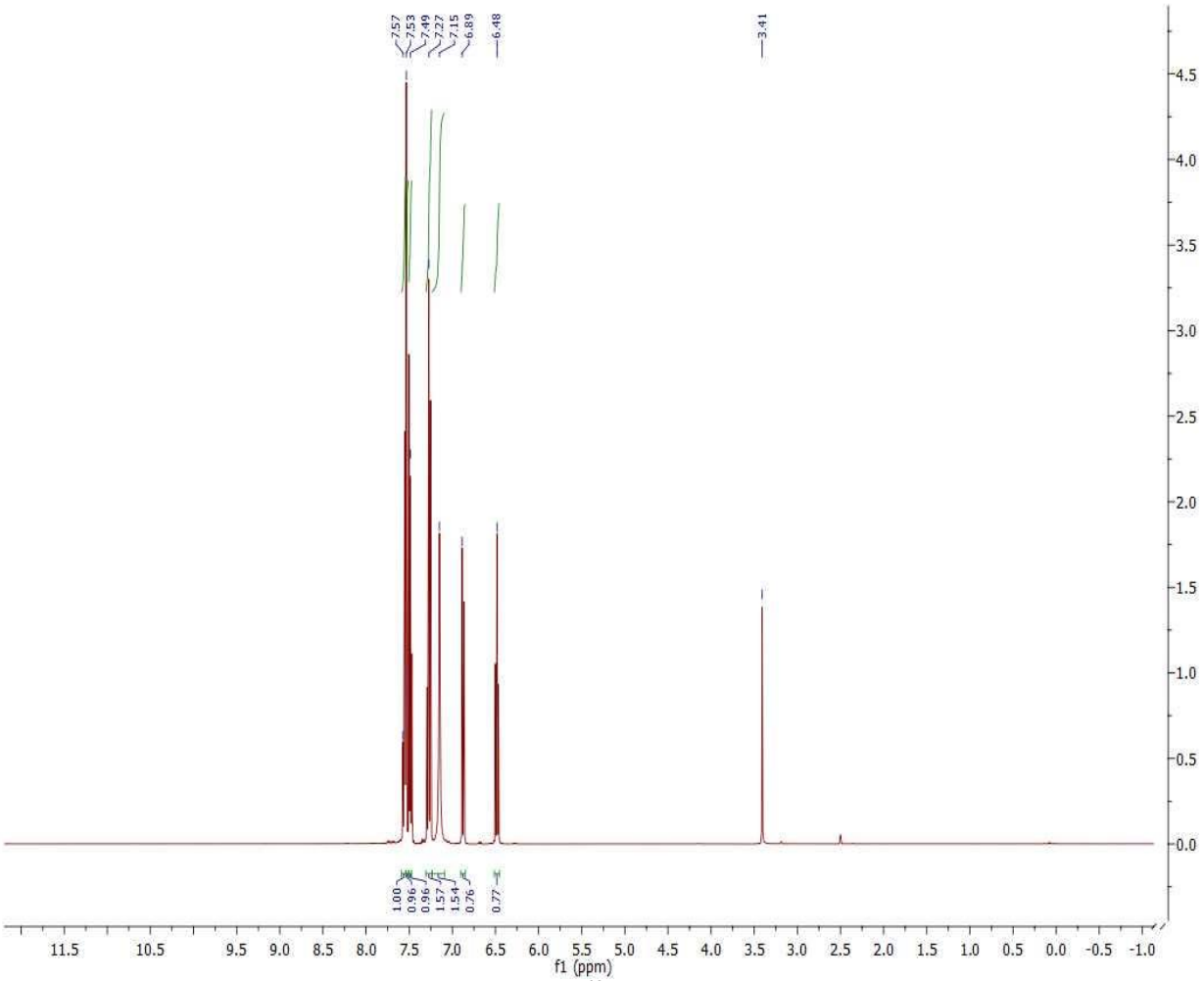

Figure 2: Carbon-13 Nuclear Magnetic Resonance (^{13}C NMR) Analysis

The ^{13}C NMR spectrum of TBF-19 revealed thirteen carbon signals, aligning with the molecular formula $C_{13}H_{11}NO$. A significantly deshielded resonance at δ 198.68 ppm confirmed the presence of a carbonyl (C=O) carbon typical of aryl ketones. Additional peaks appeared between δ 114.85–152.67 ppm, representing carbons in aromatic and amino-substituted benzene rings.

The most downfield signal (δ 152.67 ppm) was attributed to the carbon directly bonded to the amine group (C-2), while carbons within δ 128–140 ppm corresponded to aromatic ring carbons adjacent to the carbonyl linkage. This spectral profile confirms a conjugated aromatic framework typical of benzophenone derivatives (Patel *et al*., 2024; Shukla *et al*., 2025).

Table 1: Spectroscopic Data of TBF-19 in DMSO-d_6

TBF-19 (2-aminobenzophenone)		
S/N	**^{13}C**	**$^{a}\delta_H$ (mulit. *J* in Hz)**
1	117.64	--
2	152.67	--
3	117.06	6.89 (1H,s)
4	134.62	7.53 (1H,dd)
5	114.85	6.48 (1H,dd)
6	139.47	7.57 (1H,d)
7	198.68	--
8	140.75	--
9	129.28	7.27 (1H,d)
10	128.94	7.15 (1H,dd)
11	131.61	7.49 (1H,dd)
12	128.94	7.15 (1H,dd)
13	129.28	7.27 (1H,d)
		-NH_2 3.41(2H,s)

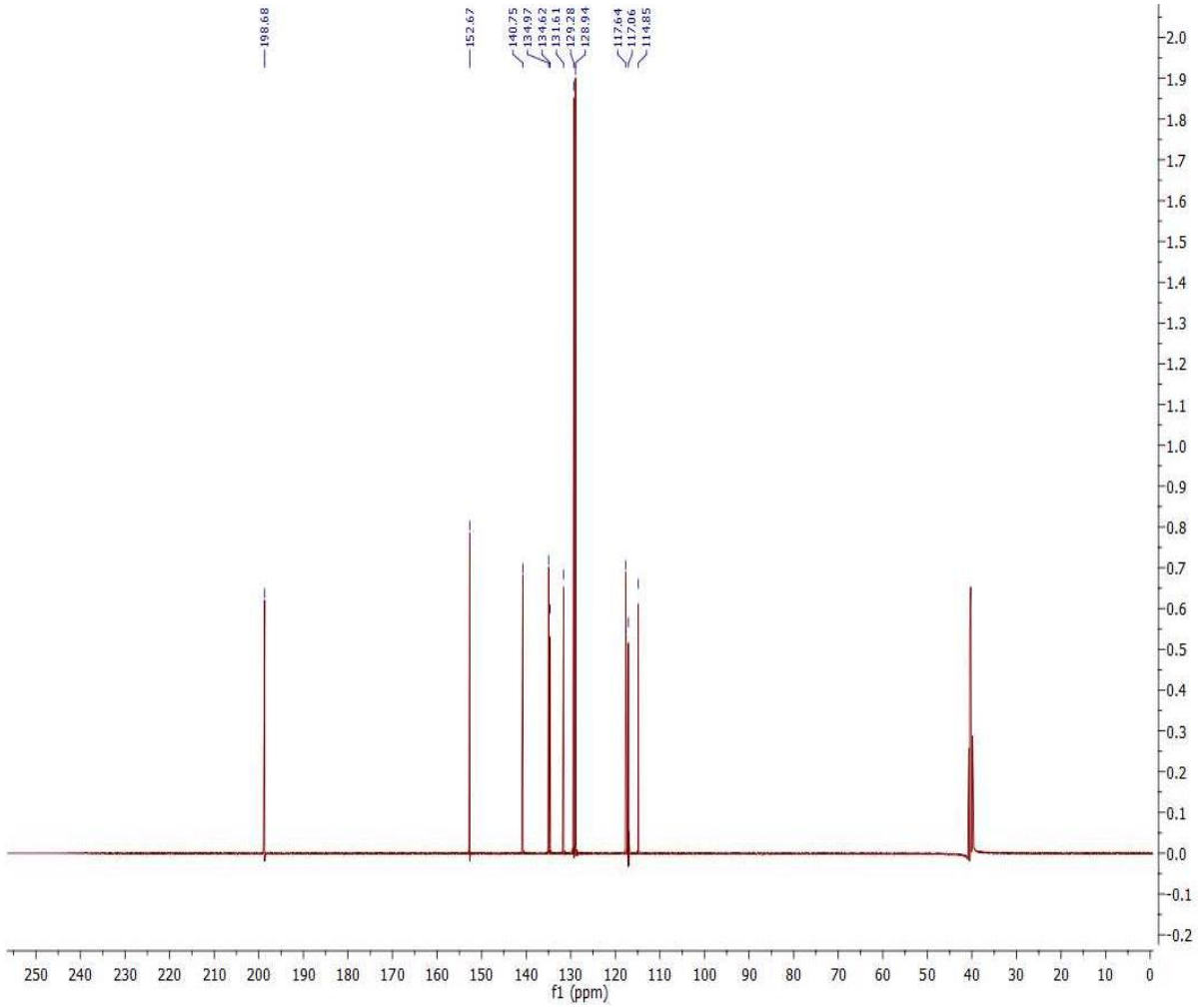

Figure 3: ^{13}C NMR (500 MHz, (DMSO-d_6) Spectrum for TBF-19

Fourier Transform Infrared (FT-IR) Spectroscopy

The FT-IR spectrum of TBF-19 corroborated the functional groups inferred from HRMS and NMR analyses. Broad absorption bands at 3428 cm^{-1} and 3312 cm^{-1} were attributed to N–H stretching vibrations of the amine (-NH_2) group. A strong absorption band at 1623 cm^{-1} was characteristic of the C=O stretching vibration in a conjugated benzophenone moiety. Additional signals

observed at 1449 cm^{-1} represented C=C stretching within aromatic rings, while absorptions at 3047 cm^{-1} and 921 cm^{-1} indicated aromatic C–H stretching and bending, respectively. The combination of these features provides compelling evidence of a conjugated aromatic ketone system bearing an amino substituent (Tarmoul *et al*., 2025; Khalid *et al*., 2024).

Structural Confirmation

Integrating the evidence from HRMS, ^{1}H NMR, ^{13}C NMR, and FT-IR, TBF-19 was unequivocally identified as 2-aminobenzophenone. The HRMS established its molecular composition, while the NMR spectra validated the presence of aromatic and amine protons and carbons consistent with an aminophenyl-benzoyl framework. FT-IR analysis further supported the existence of amine and carbonyl functionalities. Collectively, these results affirm that TBF-19 contains a substituted benzophenone skeleton with a carbonyl group conjugated to two aromatic rings and an ortho-amino substituent, a structure consistent with naturally occurring phenolic ketones exhibiting bioactive potential (Kaban *et al*., 2024; Eroğlu & Valiyeva, 2025).

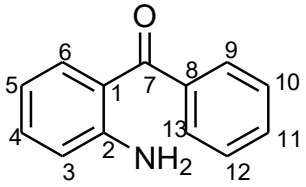

Figure 4: 2-aminobenzophenone (TBF-19)

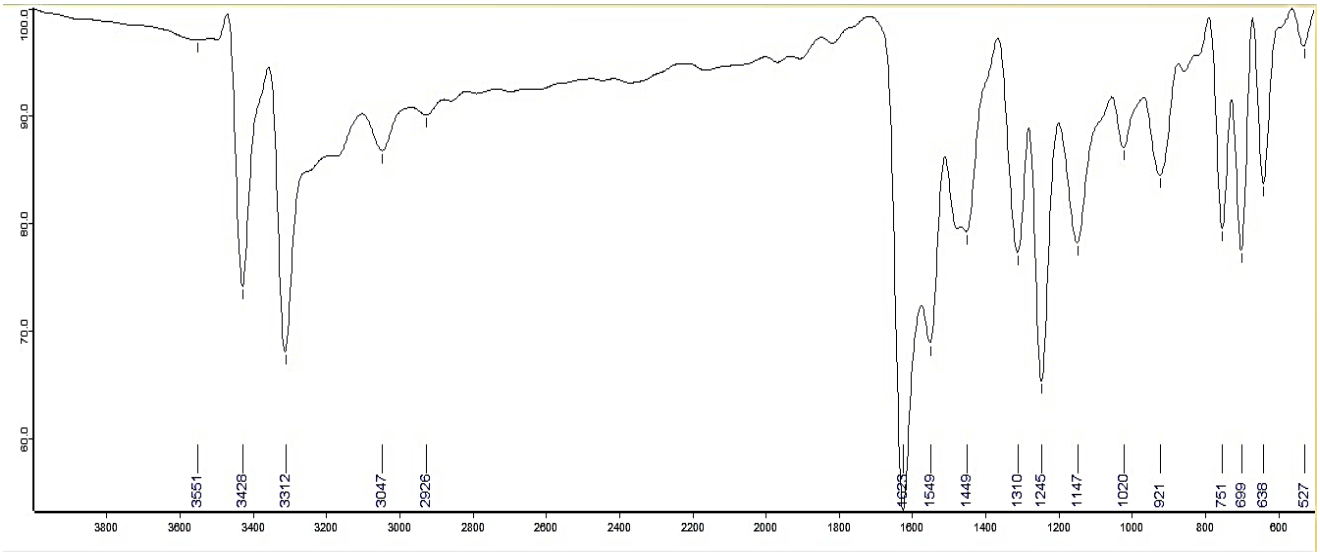

Figure 5: FT-IR Spectrum for TBF-19

Wound healing activity

Table 2: Effect of TBF-19 on the wound contraction (N = 5)

Post wounding (days)	Wound area (mm^2) (mean ± S.E.M)		
	Control	**Standard**	**TBF-19**
0	400 ± 0.00	400 ± 0.00	400 ± 0.00
4	302.31 ± 3.52	272.74 ± 4.96	276.86 ± 23.89
8	212.75 ± 2.34	152.87 ± 2.71	160.11 ± 1.14
12	64.02 ± 3.85	43.97 ± 1.36	19.18 ± 1.57
16	23.35 ± 3.62	21.00 ± 1.52	0.00 ± 0.00

The data are mean value ± standard deviation of three replicates ($P < 0.05$)

The data in Table 2, alongside the percentage contraction bar chart in Figure 6, illustrates the progression of wound healing across all groups (control, standard, and TBF-19). Wound contraction, a critical marker of healing, revealed significant differences among the control, standard, and TBF-19 groups. To ensure consistency, all groups started with an initial wound area of 400 mm^2, providing a standardized baseline for comparison. By day 4, variations in healing became evident. The control group showed the slowest progress, with a wound area of 302.31 ± 3.52 mm^2

(24.42 % contraction). The standard treatment performed better, reducing the wound area to 272.74 ± 4.96 mm^2 (31.82 % contraction), and the TBF-19 (Isolated compound) at 276.86 ± 23.89 mm^2 (30.79 % contraction). Figure 6 visually emphasizes this trend, with TBF-19 demonstrating the highest percentage contraction by day 4.

As the healing process continued, the differences became even more pronounced. By day 8, the control group had achieved a contraction of 46.81 % (212.75 ± 2.34 mm^2), while the standard treatment reached 61.78 % (152.87 ± 2.71 mm^2) and TBF-19 at 59.98 % (160.11 ± 1.14 mm^2). By day 12, the control group reached an 84 % contraction (64.02 ± 3.85 mm^2), while the standard treatment achieved 89 % (43.97 ± 1.36 mm^2) and the TBF-19 continued to lead with a remarkable contraction of 95.20 % (19.18 ± 1.57 mm^2) almost approaching complete healing. The bar chart clearly depicts these trends, highlighting the superior performance of the Isolated compound in promoting early and efficient wound closure. By day 16, the TBF-19 achieved complete wound healing (100 % contraction), meanwhile, the control and standard groups showed residual wound areas of 23.35 ± 3.62 mm^2 and 21.00 ± 1.52 mm^2, corresponding to 94.17 % and 94.75 % contraction, respectively. Their contraction comes into final completion on day 18 for standard group and between days 20-22 for the control group (figure 6).

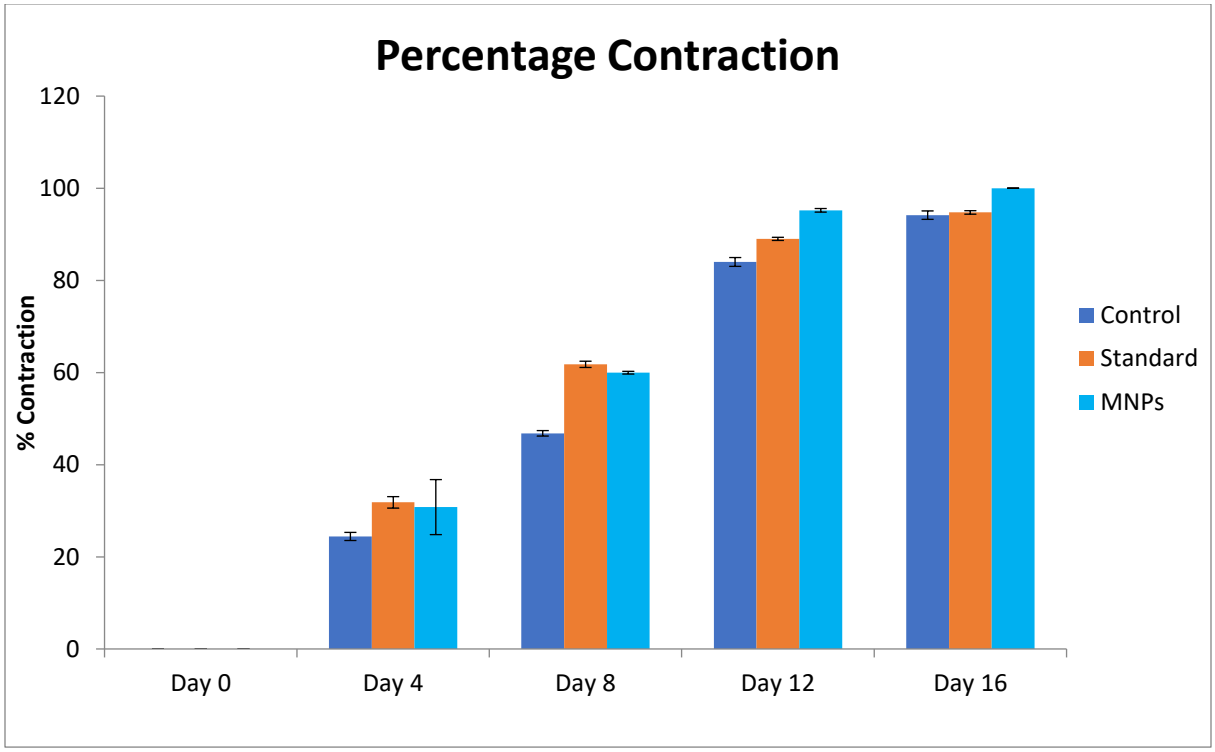

Figure 6: Bar chart showing the percentage contraction of the TBF-19 (Isolated compound), Standard (Povidone Iodine), and Control (Petroleum Jelly Lotion)

Morphological Progression of the Wound Healing

Figure 7 visually demonstrates the rapid and consistent wound closure achieved by the various groups, with TBF-19 leading the way. These findings underscore the exceptional healing potential of *Jatropha* based isolated compound, which consistently achieved faster and more effective wound contraction compared to the controls. The properties of the potent bioactive compound of *Jatropha* (TBF-19), including its antimicrobial, anti-inflammatory, and collagen-stimulating effects, likely contributed to its superior performance.

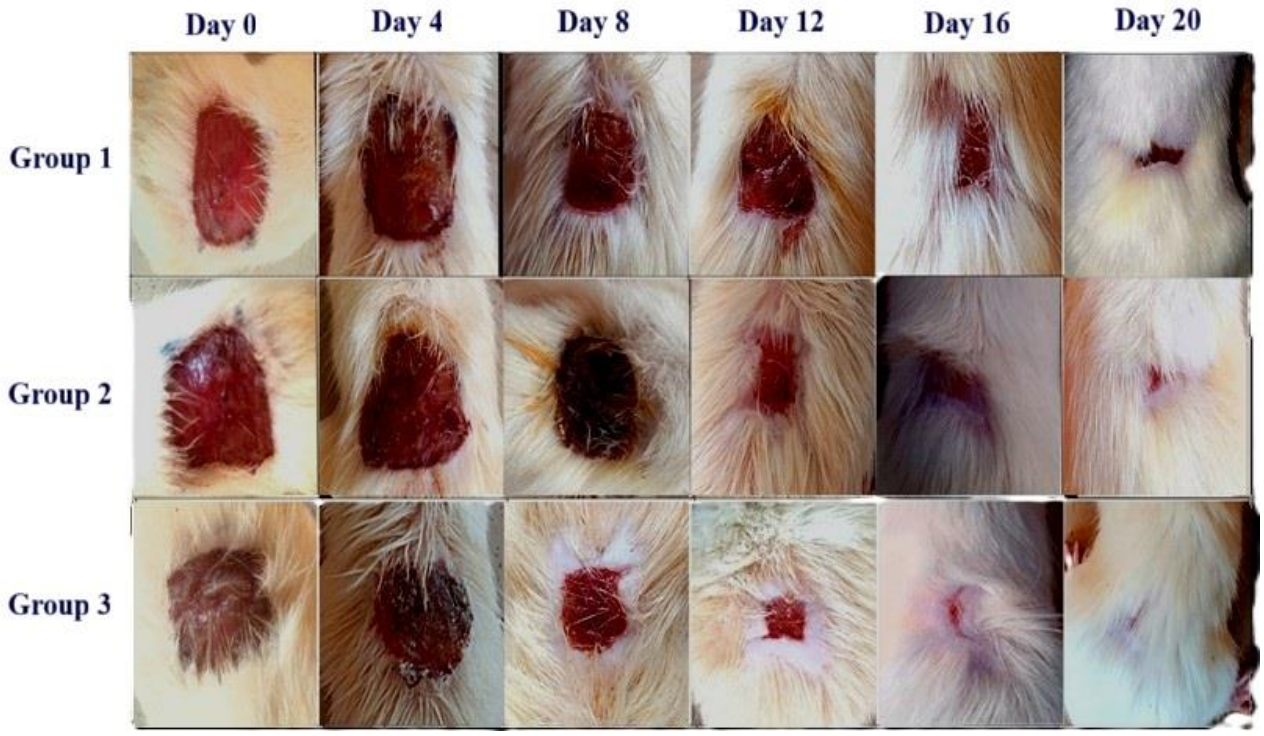

Figure 7: Representative images showing morphological changes of different experimental wound healing

The morphological progression of wound healing over 20 days in the different groups is summarized in Figure 7, showcasing the effects of various treatments. At the start (Day 0), all groups had comparable wound sizes, ensuring uniformity in the inflicted excision wounds. By Day 4, differences in healing became physically evident. The control group exhibited minimal scab formation, with pronounced inflammation and delayed contraction. The standard treatment group showed noticeable improvement, including reduced inflammation and thicker scabs. The isolated compound group (TBF-19) displayed more significant wound contraction and scab formation. By Day 8, the control group's wound remained large, the standard group showed partial re-epithelialization and reduced wound size, and TBF-19 group continued to lead with rapid contraction, thinner scabs, and early re-epithelialization. On Day 12, the control group still show slower healing progress. The standard group displayed significant wound closure and advanced re-epithelialization. TBF-19 treated wounds showed skin re-epithelialized with slightly larger wound remnants. By Day 16, the control group exhibited residual wounds with delayed healing, while the standard group achieved near-complete healing, leaving minor scarring, while the TBF-19 treated group achieved full wound closure with minimal scarring. By Day 20, the TBF-19 group achieved complete wound healing with no visible scarring, underscoring the effectiveness of *Jatropha*-based isolated TBF-19 treatment. Meanwhile the standard group also achieved full healing but with mild residual scarring and the control group, displayed delayed healing and incomplete re-epithelialization with visible scar tissue.

Epithelialization period

Figure 8 highlights the time needed for falling of eschar without any residual raw wound, (epithelialization period). Due to the long healing period of the control group, it is expected to have a longer epithelialization period in comparison with the standard, and the TBF-19 group. The according to the bar chart in figure 8, TBF-19 stood out with the shortest epithelialization period, indicating the fastest restoration of the skin barrier compared to both the control and standard groups, emphasizing the effectiveness of isolated compound in speeding up wound healing.

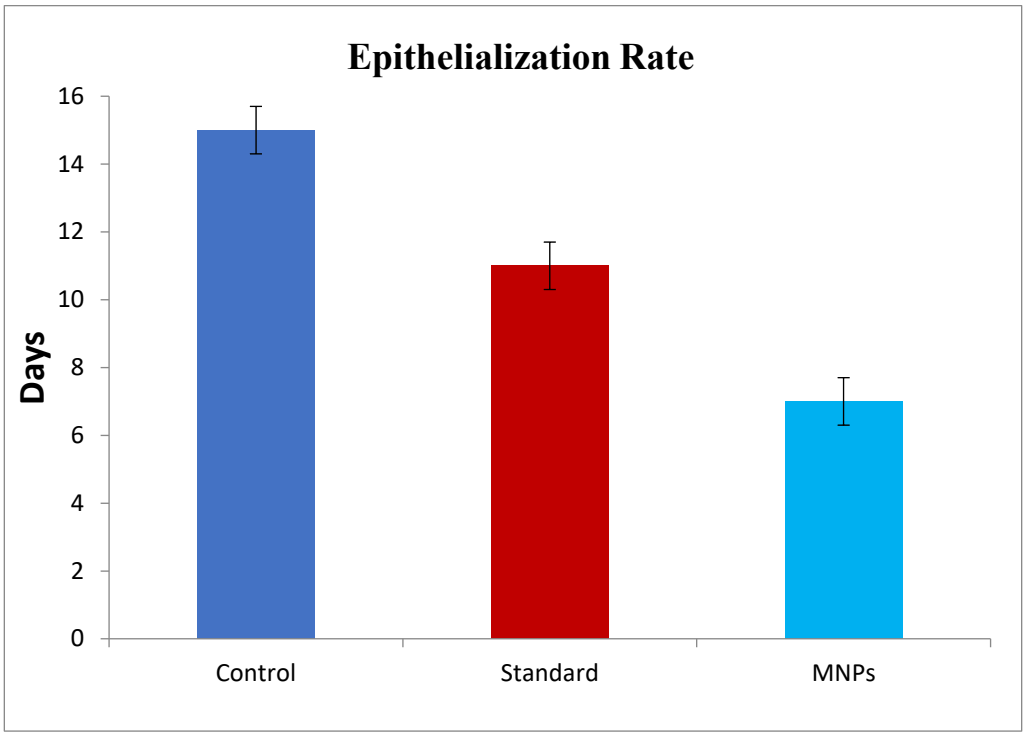

Figure 8: Epithelialization Period of the TBF-19 (Isolated compound), Standard (Povidone Iodine), and Control (Petroleum Jelly Lotion)

Total Protein Analysis and Hydroxyproline Content

Figure 9 highlights the total protein content in healing tissues, offering valuable insight into the process of tissue repair and regeneration. The control group had the lowest protein levels, reflecting slower cellular repair and the formation of granulation tissue. The standard treatment showed moderate improvement, suggesting better tissue regeneration compared to the control. The extract treatments, however, significantly boosted protein levels, TBF-19 has the highest protein content. This pattern is consistent with the hydroxyproline data (Table 3), further confirming that TBF-19 promote more effective tissue repair and protein synthesis during healing. The higher protein levels observed with TBF-19 are linked to their ability to enhance collagen deposition and accelerate wound contraction, highlighting their superior healing potential.

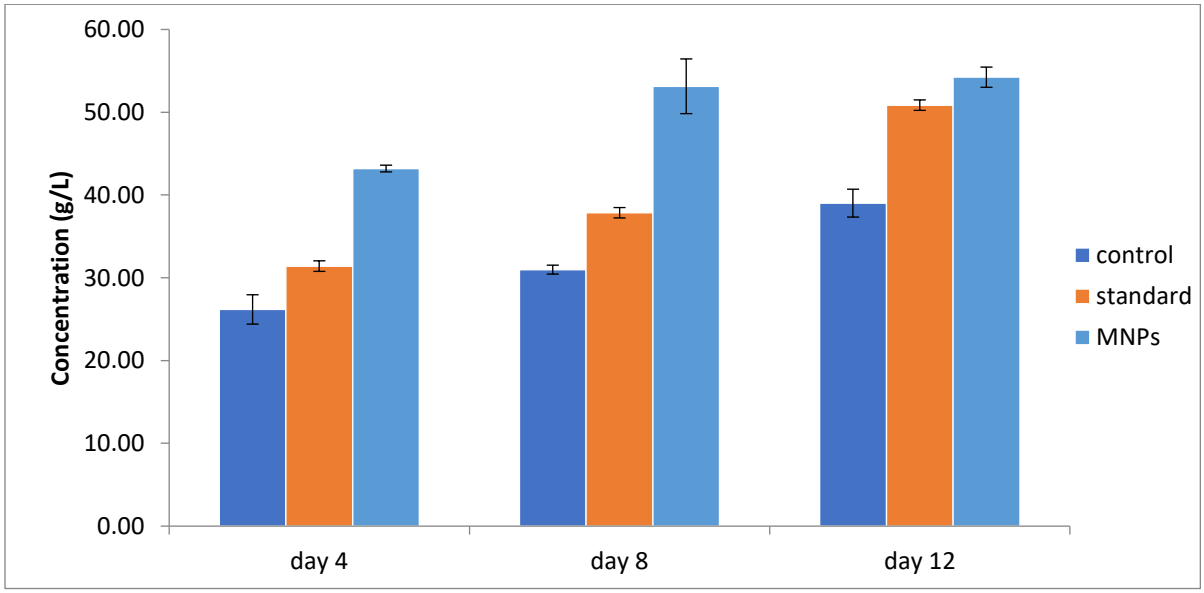

Figure 9: Total Protein Analysis of the Healing Tissues

The data in Table 3 reveals notable differences in hydroxyproline content across the treatment groups. The control group showed the lowest levels (5.2 ± 0.30 mg/g tissue), the standard treatment improved hydroxyproline levels to 7.8 ± 0.40 mg/g tissue, reflecting a moderate boost in collagen synthesis and the TBF-19 stood out with the highest hydroxyproline content (12.50 ± 1.50 mg/g tissue). This result highlights the superior ability of TBF-19 to enhance collagen production, a critical factor in wound repair. These findings suggest that the bioactive components in *Jatropha* nanoparticles strongly promote collagen synthesis, contributing to their exceptional wound-healing effectiveness.

Table 3: Hydroxyproline content of granulation

Groups	Hydroxyproline Content (mg/g tissue)
Control	5.2 ± 0.30
Standard	7.8 ± 0.40
TBF-19	12.50 ± 1.50

The data are mean value ± standard deviation of three replicates ($P < 0.05$)

Discussion

Wound healing is a highly intricate biological process that involves a well-coordinated series of cellular and biochemical events to repair damaged tissue. It progresses through three overlapping stages: inflammation, proliferation, and remodeling (Goyal *et al*., 2024). During the inflammatory phase, the process begins with hemostasis to stop bleeding, followed by the arrival of neutrophils to fight infection and clear debris. This phase sets the stage for the formation of granulation tissue, a critical component that marks the transition to the proliferation phase (Goyal *et al*., 2024). The data in Table 2, along with the accompanying charts and images (figure 6 and 7), clearly indicate that the test formulation containing TBF-19 promoted a faster and more effective wound contraction compared to both the standard and negative control groups. These phytoconstituents exhibit anti-inflammatory, antioxidant, and antimicrobial activities, all essential for tissue regeneration. Flavonoids and saponins, in particular, promote cellular proliferation, angiogenesis, and collagen synthesis, while reducing inflammation and oxidative stress, thereby accelerating wound closure and epithelialization (Tinpun *et al*., 2020; Salim *et al*., 2018; Khalifa *et al*., 2021; Mule *et al*., 2024; Inya & Mesak, 2024; Carvalho *et al*., 2021; Vitale *et al*., 2022).

Topical formulations of *Jatropha curcas* latex and leaf extracts contains 2-Aminobenzophenone (TBF-19), a derivative of alkaloids, specifically belonging to the class of quinazoline alkaloid precursors or anthranilic acid derivatives significantly increased wound contraction, collagen deposition, and granulation tissue strength in rat and mouse models, with effects comparable to standard treatments (Tinpun *et al*., 2020; Shetty *et al*., 2006; Mohammed *et al*., 2022; Sachdeva *et al*., 2011; Odoh *et al*., 2011; Inya & Mesak, 2024). Literatures reviewed that *Jatropha*-based creams and gels reduced leukocyte infiltration and inflammation, promoted neovascularization, and enhanced re-epithelialization during the wound healing process (Salim *et al*., 2018; Risky *et al*., 2021; Bahittah *et al*., 2023; Sitorus *et al*., 2021; Inya & Mesak, 2024). Another In vitro study carried that by Tinpun and Khalifa showed that *Jatropha* extracts stimulate fibroblast proliferation and collagen production without cytotoxicity, supporting their role in tissue remodeling (Tinpun *et al*., 2020; Khalifa *et al*., 2021).

The complex relationship between epithelialization, total protein levels, and hydroxyproline plays a pivotal role in determining the effectiveness of wound healing. Each of these factors contributes uniquely to the repair process, working together to ensure proper tissue regeneration and restoration of the skin's protective barrier. Epithelialization, which involves the migration of keratinocytes, is a fundamental step in wound closure. This process is essential for restoring the epidermal barrier and is heavily influenced by factors such as moisture levels, which facilitate cell movement and improve re-epithelialization (Tan and Dosan, 2019). The activity of keratinocytes is further stimulated by cytokines and growth factors like epidermal growth factor (EGF) and transforming growth factor-beta (TGF-β), which enhance their proliferation and migration (Koivisto *et al*., 2011). These factors underscore the critical importance of a supportive wound environment to optimize epithelialization.

Hydroxyproline is a direct indicator of collagen content and turnover, reflecting the strength and integrity of newly formed tissue during wound healing. The biochemical assays presented in the hydroxyproline and total protein figures revealed higher values in the TBF-19-treated group compared to other groups, implying an increased rate of collagen biosynthesis and extracellular matrix stabilization. Hydroxyproline, a key indicator of collagen turnover, directly reflects the strength and integrity of the newly formed tissue. The superior performance of the TBF-19 group in these biochemical parameters aligns with the observations of Ustuner *et al*. (2019), Chen *et al*. (2019), and Mathew-Steiner *et al*. (2021), who emphasized that

plant-derived bioactive molecules, increased hydroxyproline levels, as seen in the TBF-19-treated group, signify accelerated collagen biosynthesis and improved extracellular matrix (ECM) stabilization, key for robust wound closure and tissue remodeling. The elevated protein content also implies active cellular metabolism and faster granulation tissue formation, which are hallmarks of effective wound healing (Chen *et al*., 2019; Ustuner *et al*., 2019).

Total protein levels also serve as an important indicator of overall wound-healing capacity. Proteins are vital for various aspects of tissue repair, including cell proliferation, matrix deposition, and enzymatic activity. Increased total protein levels reflect an active synthesis of the essential components needed for regeneration (Lipatov *et al*., 2024). However, disruptions such as chronic inflammation or infection can hinder protein synthesis, epithelialization, and collagen formation, leading to delayed healing and potential complications.

While the interplay between epithelialization, hydroxyproline, and total protein levels highlights their collective importance in wound healing, challenges remain in optimizing these processes. Total protein analysis, particularly through the quantification of hydroxyproline, serves as a critical marker in assessing wound healing efficacy. Hydroxyproline, a major component of collagen, correlates strongly with the maturation of connective tissue in healing wounds, as demonstrated in studies where increased hydroxyproline levels were associated with reduced wound area and enhanced scar formation (Lipatov *et al*., 2024). Additionally, biochemical assays for hydroxyproline have been validated against histomorphometric analyses, confirming their reliability in measuring collagen content in skin biopsies (Caetano *et al*., 2016).

CONCLUSION

The present study successfully isolated and identified 2-aminobenzophenone (TBF-19) from Jatropha curcas leaves and demonstrated its potent wound-healing activity in vivo. The compound significantly accelerated wound contraction, shortened epithelialization time, and enhanced collagen deposition, outperforming both the negative control and standard treatment. These findings validate the ethnomedicinal use of J. curcas and highlight TBF-19 as a promising bioactive candidate for topical wound therapeutics. Further studies should explore structure-activity relationships and clinical translation of this natural product.

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