

An Open Access Peer Reviewed Journal

International Journal of Pharmacy & Pharmaceutical Sciences



Editor-in-Chief

Prof. M. S. Bhatia, India
(Bharati Vidyapeeth College of Pharmacy, Kolhapur, India)
Email: manish.bhatia@bharatividyapeeth.edu
Email: editor@ijppsjournal.com

Associate Editors

Dr. Avijeet Jain, India
(Shri Sathya Sai Institute of Pharmaceutical Sciences, Bhopal, India)
Email: avijeet_9826275340@rediffmail.com

Diah Ayu Maharani
Department of Preventive and Public Health, Dentistry Faculty of Dentistry, University of Indonesia
Email: raniabdillah@gmail.com

Dr. Subhash C Mandal, India
(Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India)
Email: subhashmandal@yahoo.com

Dr. Lokesh Deb, India
(Medicinal Plants and Horticultural Resources Division, Institute of Bioresources and Sustainable Development (IBSD), Department of Biotechnology, Government of India, Takyelpat Institutional Area, Imphal, Manipur, India)
Email: lokesh_deb@rediffmail.com

Dr. Wong Tin Wui, Malaysia
(Non-Destructive Biomedical and Pharmaceutical Research Center, University of Technology MARA, Malaysia)
Email: wongtinwui@yahoo.com

Assistant Editor

Dr. Idress Hamad Attittala, Libya
(Omar El-Mukhtar University, Faculty of Science, Botany Department, El-Beida, Libya)
Email: idressattittalla2004@yahoo.com

Dr. Alok Nahata, Malaysia
(Alor Star, Malaysia)
Email: aloknahata@gmail.com

Executive Editor

Mr. Niranjan Pathak, India
(Dept. of Pharmaceutical Sciences, Dr H S Gour Central University, Saugor, India)
Email: niranjanpathaklib@gmail.com

Editorial Board Members

Dr. Furhan Iqbal
Bahauddin Zakariya University Multan, Pakistan

Dr. Ebtessam Ahmed Mohammed Essa
Department of Pharmaceutical Technology, Faculty of Pharmacy, Tanta University, Tanta, Egypt

Dr. Syed Muhammad Farid Hasan
Faculty of Pharmacy, University of Karachi, Karachi, Pakistan

Dr. Abdel Raheim Mohammed Ahmed Donia
College of Pharmacy – Salman Bin Abdul Aziz University, Egypt

Dr. Pranay Jain
Institute of Engineering & Technology, Kurukshetra University, Kurukshetra, Haryana, India

Dr. Dr. Niaz Ali
Institute of Basic Medical Sciences, Khyber Medical University, Peshawar, Pakistan

Dr. M. Saeed Arayne
Chairman, Department of Chemistry, University of Karachi, Pakistan

Dr. Wanzala Wycliffe
School of Pure and Applied Sciences, South Eastern Kenya University, Kenya

Dr. Mayuree Tangkiatunjai
Faculty of Pharmacy, Srinakharinwirot University, Ongkharak, Nakhonnayok, Thailand

Dr. Javed Intekhab
G. F. College (Rohilkhand University), Shahjahanpur, U.P., India

Dr. Manish P. Patel
Dept. of Pharmaceutics and Pharmaceutical Technology, Nootan Pharmacy College, Visnagar, Gujarat, India

Dr. Narendra Babu Shivanagere Nagojappa
J.N. Medical College, KLE University, Belgaum, Karnataka, India

Dr. C. Chellaram
Vel Tech Multi Tech Engg. College, Chennai, India

Dr. Mehdi Shafiee Ardestani
Department of Medicinal Chemistry and Radiopharmacy, Tehran University of Medical Sciences, Tehran, Iran

Dr. Amal Amin Mohamed
Plant Biochemistry Department; Agriculture Division -National Research Center, Dokki, Cairo, Egypt

Dr. Rabab Kamel Mahmoud
National Research Center, Cairo, Egypt

Dr. Syed Adnan Ali Shah
Universiti Teknologi MARA (UiTM), Puncak Alam Campus, Bandar Puncak Alam, Selangor D. E., Malaysia

Dr. Sat Pal Singh Bisht
Roland Institute of Pharmaceutical Sciences Berhampur, Orissa, India.

- Dr. Anup Naha
Dept. of Pharmaceutic, MCOPS, Manipal, Karnataka, India
- Dr. Jagdish Labhubhai Kakadiya
Indubhai Patel College of Pharmacy and Research Centre, Petlad-Khambhat Road, Dharmaj, Anand, Gujarat, India
- Dr. Maha Ali Eissa Ahmed
Department of Pharmacology, Faculty of Pharmacy, MISR University for Science and Technology (MUST), Giza Governorate, Egypt.
- Dr. Rajesh Mukthavaram
Health Science Drive, University of California, San Diego, Lajolla, CA, California, USA
- Dr. Saifullah Khan
International Islamic University, Malaysia
- Norhaniza Aminudin
Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia.
- Dr. Shazia Jamshed
Kulliyah of Pharmacy, International Islamic University Malaysia (IIUM), Kuantan, Pahang, Malaysia
- Dr. Mayuree Tangkiatkumjai
Department of Clinical Pharmacy and Social Pharmacy Faculty of Pharmacy, Srinakharinwirot University, Nakhonnayok, Thailand
- Dr. İsmail Murat Palabiyik
Faculty of Pharmacy, University of Ankara, Tandoğan, Ankara, Turkey
- Nadeem A. Kizilbash
Faculty of Medicine, Northern Border University Arar, Saudi Arabia
- Dr. Debajit Kalita
Department of Botany, Morigaon College, Assam, India
- Dr. Seyed Mohammad
Department of Biology, Faculty of Basic Sciences, University of Mazandaran, Babolsar, Iran
- Dr. Shalini Sivadasan
Faculty of pharmacy, AIMST University, Semeling, Kedah, Malaysia
- Dr. Sujimon Tanvichien
Srinakharinwirot University Nakornayok Rd. A. Ongkarak T.Ongkarak, Nakornayok, Thailand
- Prof. Dr.-Ing. habil. Dr. h. c. Lothar Mörl
Institute für Apparate- und Umwelttechnik Otto-von-Guericke-Universität Magdeburg, Germany
- Dr. V. Ravichandran
Faculty of Pharmacy, AIMST University, Semeling, Kedah, Malaysia
- Dr. Zahid Hussain
Department of Pharmaceutics, Faculty of Pharmacy, Universiti Teknologi MARA, Puncak Alam Campus, Malaysia
- Dr. Ajay Kumar Meena
Department of AYUSH, Ministry of Health & Family Welfare, Government of India, India
- Dr. Ashish C. Suthar
Herbal R & D, Piramal Life Sciences Ltd., Mumbai, India
- Dr. Manish A. Rachchh
Pharmacological Research and IPR University road, Rajkot, Gujarat, India
- Kiran Kumar Chereddy
Manager at Novartis Pharma AG
Postfach 4002, Basel
Switzerland
- Dr. Fahd M. Abd Al Galil
Department of Zoology, Faculty of Applied Science, Tamar University, Yemen
- Dr. Priyanka Bhatt
Department of Pharmaceutical Sciences, College of Pharmacy, University of South Florida, USA
- Dr. Beril Anilanmert
Istanbul University-Cerrahpasa Institute of Forensic Sciences, Cerrahpasa/Istanbul
- Dr. Shahu Ingole
Smt. Kashibai Navale Medical College & Hospital, Pune India
- Dr. Gina Samy El-Feky
Pharmaceutics Department, Faculty of Pharmacy, Modern Science and Arts University, Egypt
- Dr. Abdalla Ahmed Elbashir Ahmed
Khartoum University, Sudan
- Dr. Yesudass Dominic Ravichandran
School of Advanced Sciences, VIT University, Vellore, Tamil Nadu, India
- Dr. Seema Akbar
Research Institute of Unani Medicine, The University of Kashmir Campus, Srinagar, J. & K., India
- Dr. P. Thillai Arasu
Department of Chemistry, Wollega University, Nekemta, Ethiopia
- Dr. Sooraj S. Nath
Safi Institute of Advanced Study, Kozhikode, India
- Dr. Erum Shireen
Dept. of Biochemistry, University of Karachi, Pakistan
- Dr. M. M. Gupta
School of Pharmacy, Faculty of Medical Sciences, The University of The West India, India
- Dr. Najma Sultana
United Biotechnologies, Karachi, Pakistan
- Dr. Sivakumar P
Department of Petroleum Engineering, JCT College of Engineering and Technology, Pichanur, Tamil Nadu, India
- Dr. Evren Algin Yapar
Department in Ministry of Health, Turkish Medicines and Medical Devices Agency, Ankara, Turkey
- Dr. Vishal Vijay Pandey
Jaywantrao Sawant College of Pharmacy & Research, Hadapsar, Pune, India
- Dr. Shamkuwar Prashant Babarao
Government College of Pharmacy, Thiba Palace, Ratnagiri, India
- Dr. S. K. Starling
Department of Chemistry, Mewar University Chittorgarh, Rajasthan, India
- Dr. Syed Sajjad Hussen
Manipal College of Pharmaceutical Sciences, Manipal University, India
- Dr. Ahmed Osman
Department of Psychology, Faculty of Education, Assiut University, Malaysia
- Dr. Abdel-Tawab Halim Mossa Abd El-Aziz
Department of Pesticide Chemistry, National Research Centre (NRC) Dokki, Cairo, Egypt
- Dr. V. Vinod Prabhu
Department of Biochemistry, University of Madras, Guindy campus, Tamil Nadu, India
- Dr. Ching Siew Mooi
University Putra, Malaysia
- Dr. Asif Husain
Jamia Hamdard University, New Delhi, India
- Dr. Muhammad Shahzad Aslam
School of Bioprocess Engineering, Universiti Malaysia Perlis, Kompleks Pusat Pengajian Jejawi, Arau, Perlis
- Dr. Gláucio Diré Feliciano
State University Center Foundation of the West Zone / Rio de Janeiro, Brazil
- Dr. Dilipkumar Pal
Department of Pharmaceutical Sciences, Guru Ghasidash Vishwavidyalaya, Bilaspur, C.G., 495 009, India
- Ali Abdullah Ali Al-yahawi
Assistant Professor of Clinical Pharmacy & Therapeutics, Yemen

Original Article(s)

- [SIMULTANEOUS ESTIMATION OF MOMETASONE FUROATE AND FORMOTEROL FUMARATE BY HPLC METHOD IN ROTACAPS](#)
 Aarti S. Zanwar, Dhanya B. Sen, Ashim Kumar Sen, A. K. Seth
 Pages 12-16

 - [View PDF Abstract Download PDF](#)
 - [View HTML](#)
- [EVALUATION OF BACTERICIDAL ACTIVITY OF AN ANTISEPTIC GEL THROUGH THE DILUTION-NEUTRALIZATION METHOD](#)
 Janeth Del Carmen Arias-Palacios, LIBARDO HERNANDEZ ESQUIVEL, Dorelly Sandoval Medina, Tatiana Diaz B.
 Pages 17-20

 - [View PDF Abstract Download PDF](#)
 - [View HTML](#)
- [EVALUATION OF ANTIOXIDANT AND CHEMOPREVENTIVE POTENTIAL OF METHANOLIC EXTRACTS OF LEAF OF AEGLEMARMELOS ATTRIBUTES TOWARDS DUCTAL CARCINOMA STUDIED IN MCF7 CELLS](#)
 B. Arirudran, B. Janani, U. S. Mahadeva Rao
 Pages 21-25

 - [View PDF Abstract Download PDF](#)
 - [View HTML](#)
- [ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF CURCUMIN AND CYCLOSPORINE BY RP-HPLC](#)
 Neha Desai, Munira Momin, Upasana Singh, Tabassum Khan, Atul Sherje
 Pages 26-33

 - [View PDF Abstract Download PDF](#)
 - [View HTML](#)
- [AN INNOVATIVE METHOD DEVELOPMENT AND FORCED DEGRADATION STUDIES FOR SIMULTANEOUS ESTIMATION OF SOFOSBUVIR AND LEDIPASVIR BY RP HPLC](#)
 B. Anjaneyulu Reddy, Md. Irshad Alam, Nazia Khanam, P. R. Adhkrishnanand
 Pages 34-41

 - [View PDF Abstract Download PDF](#)
 - [View HTML](#)
- [ANTISICKLING ACTIVITY EVALUATION OF FRACTIONS OBTAINED FROM WHOLE EXTRACTS OF NEWBOULDIA LAEVIS P. BEAUV \(BIGNONIACEAE\)](#)
 Affo Dermane, Kafui Kpegba, Kossi Metowogo, M. Kossi Joppa, A. Kodjo Aklidikou
 Pages 42-46

 - [View PDF Abstract Download PDF](#)
 - [View HTML](#)
- [THE ENHANCEMENT OF COLLAGEN SYNTHESIS PROCESS ON DIABETIC WOUND BY MERREMIA MAMMOSA \(LOUR.\) EXTRACT FRACTION](#)
 Ancah Caesarina Novi Marchianti, Mega Citra Prameswari, Elly Nurus Sakinah, Evi Umayah Ulfa
 Pages 47-50

 - [View PDF Abstract Download PDF](#)
 - [View HTML](#)
- [ALUMINA CATALYST: SYNTHESIS OF NOVEL QUINAZOLINE DERIVATIVES AND THEIR SOLUBILITY INCREASES THROUGH INCLUSION WITH \$\beta\$ -CYCLODEXTRIN](#)
 Mossaraf Hossain, Ashis Kumar Nanda
 Pages 51-58

 - [View PDF Abstract Download PDF](#)
 - [View HTML](#)
- [MUTANT P21 PEPTIDES COULD ACT AS AN IMPROVED CYCLIN A INHIBITORS FOR CANCER THERAPY: AN IN SILICO VALIDATION](#)

- [View PDF Abstract Download PDF](#)
 - [View HTML](#)
- [ANTIOXIDANT STUDIES ON METHANOL AND AQUEOUS EXTRACTS OF GYMNOSPORIA MONTANA PLANT](#)
Nishat Ansari, Divya Chandel
Pages 65-70
 - [View PDF Abstract Download PDF](#)
 - [View HTML](#)
- [IN VITRO ANTI-INFLAMMATORY ACTIVITY OF SYRINGIC ACID](#)
Shilpee Chanda, Archana R. Juvekar
Pages 71-73
 - [View PDF Abstract Download PDF](#)
 - [View HTML](#)
- [DETERMINATION, ISOLATION, AND IDENTIFICATION OF AUCUBIN AND VERBASCOSIDE IN THE LEAVES OF IRAQI PLANTAGO LANCOLETA L. USING DIFFERENT DETECTING METHODS](#)
Hasan A. Khalaf, Ibrahim S. Abbas, Amani A. Tawfeeq, Monther F. Mahdi
Pages 74-80
 - [View PDF Abstract Download PDF](#)
 - [View HTML](#)
- [DEVELOPMENT OF A SENSOR BY ELECTRO-POLYMERIZATION OF ERICHROME BLACK-T ON GLASSY CARBON ELECTRODE AND DETERMINATION OF AN ANTI-INFLAMMATORY DRUG DICLOFENAC](#)
Rohini M. Hanabaratti, Jayant I. Gowda, Suresh M. Tuwar
Pages 81-87
 - [View PDF Abstract Download PDF](#)
 - [View HTML](#)
- [POLYPHARMACY INDUCED DRUG INTERACTIONS, ADVERSE DRUG REACTIONS \(ADR\) AND MEDICATION ERRORS IN TERTIARY CARE SOUTH INDIAN HOSPITAL](#)
Siddarama R., Bharath Naidu J., Joshiree K. P., Sahithi Lakshmi V.
Pages 88-93
 - [View PDF Abstract Download PDF](#)
 - [View HTML](#)
- [ISOLATION AND CHARACTERISATION OF RAPAMYCIN, TEMSIROLIMUS REGIO ISOMER \(MONOESTER\) AND TEMSIROLIMUS DIESTER IN TEMSIROLIMUS DRUG](#)
Gorla S. Reddy, Chava V. N. Rao
Pages 94-99
 - [View PDF Abstract Download PDF](#)
 - [View HTML](#)
- [THE BENEFICIAL EFFECT OF 20% SUNFLOWER SEED OIL CREAM ON MILD ATOPIC DERMATITIS IN CHILDREN](#)
Reiva Farah Dwiyana, Hartati Purbo Darmadji, Risa Miliawati Nurul Hidayah, Fitra Hergyana, Srie Prihantini Gondokaryono, Inne Arline Diana, Catherina Jessica Sutantoyo, Hendra Gunawan
Pages 100-103
 - [View PDF Abstract Download PDF](#)
 - [View HTML](#)
- [2D AND 3D-QSAR ANALYSIS OF AMINO \(3-\(\(3, 5-DIFLUORO-4-METHYL-6-PHENOXYPYRIDINE-2-YL\) OXY\) PHENYL\) METHANIMINIUM DERIVATIVES AS FACTOR XA INHIBITOR](#)
Smita Suhane, A. G. Nerkar, Kumud Modi, Sanjay D. Sawant
Pages 104-114
 - [View PDF Abstract Download PDF](#)
 - [View HTML](#)

Original Article

**THE ENHANCEMENT OF COLLAGEN SYNTHESIS PROCESS ON DIABETIC WOUND BY
MERREMIA MAMMOSA (LOUR.) EXTRACT FRACTION**

**ANCAH CAESARINA NOVI MARCHIANTI^{1*}, MEGA CITRA PRAMESWARI², ELLY NURUS SAKINAH³, EVI UMAH
ULFA⁴**

¹Department of Public Health, Faculty of Medicine, University of Jember, Jalan Kalimantan No.37 Jember, Indonesia, ²Medical Education Program, Faculty of Medicine, University of Jember, Jalan Kalimantan No.37 Jember, Indonesia, ³Department of Pharmacology, Faculty of Medicine, University of Jember, Jalan Kalimantan No.37 Jember, Indonesia, ⁴Department of Microbiology and Pharmaceutical Biotechnology, Faculty of Pharmacy, University of Jember, Jalan Kalimantan No.37 Jember, Indonesia
Email: ancah@unej.ac.id

Received: 06 Oct 2018 Revised and Accepted: 20 Dec 2018

ABSTRACT

Objective: This research aimed to evaluate the effect of fractionation of *Merremia mammosa* Lour. (*Mm* (Lour.)) extract on diabetic wound healing by observing the collagen synthesis process and to search the most potent fraction.

Methods: Wistar rats were divided into five groups (n=5), i.e., K-(negative control), K+(positive control), K1 (ethyl acetate fraction), K2 (water fraction), and K3 (n-hexane fraction). The *Mm* (Lour.) was extracted with ethanol 70%, then fractionated by using three solvents which have different polarity. The rats were adapted in 7 d, then induced into diabetic by streptozotocin dose 40 mg/kg body weight. The wound was made by Morton excision method. Treatment was given every two days and a skin biopsy was done on day 11. Analysis of collagen density was done by photomicrograph of histopathology preparations in Masson's trichrome stained by using trinocular microscope with 400x magnification in 6 fields of view, then processed by imageJ software and analyzed by appropriate statistic tool.

Results: The results of this research showed that fractionation of *Mm* (Lour.) extract significantly enhanced diabetic wound healing based on macroscopic (percentage of wound healing) and collagen density with p-value<0.05 when compared with negative control, especially the water fraction (p=0.000). The follow-up post hoc analysis showed that there was no significant (p=0.989) or there was no meaningful difference in the group of water fraction when compared to positive control.

Conclusion: Water fraction is the extract fraction of *Mm* (Lour.) which has the most significant influence on diabetic wound healing showed by enhancement of collagen synthesis.

Keywords: Wound healing, Diabetic wound, *Merremia mammosa* Lour, Collagen density

© 2019 The Authors. Published by InnoVare Academic Sciences Pvt Ltd. This is an open-access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>)
DOI: <http://dx.doi.org/10.22159/ijpps.2019v11i2.30170>

INTRODUCTION

Diabetes Mellitus (DM) is a chronic metabolic disease caused by the insufficient production of insulin in the pancreas or the inability of the body to effectively use insulin produced. According to the International Diabetes Federation (IDF) [1], there were 382 million people living with DM in the world by 2013 and it is estimated that this number will increase to 592 million people by 2035. As many as 25% of DM patients suffer from diabetic wounds and 85% of them experienced amputation [2]. Healing of diabetes wounds takes longer than normal wounds due to the disruption of the entire wound healing process. Therefore, proper treatment is needed. Treatment of diabetic foot commonly relies on gentamicin topical antibiotics. However, gentamicin causes side effects which can cause skin irritation, redness, allergies, and edema [3, 4].

Merremia mammosa (Lour.) (*Mm* (Lour.)) contains tannin (as antibacterial) and flavonoids (as an anti-inflammatory and antioxidant) which can inhibit bacterial growth, trigger macrophages to produce growth factors, neutralize free radicals, accelerate the inflammatory phase, trigger cell proliferation, and increase collagen synthesis which plays a role in wound closure [5, 6]. In our previous investigation [5-7] the extract used was ethanol. The investigation showed positive results on the percentage of healing and the number of fibroblasts in diabetic wounds. However, the extract did not only contain active ingredients but was still mixed with inactive components which can reduce the effectiveness of the extract. Therefore, it is necessary to do a separation to isolate multilevel compounds namely by fractionation [8].

Fractionation of *Mm* (Lour.) ethanol extract was conducted by using three different types of solvents based on polarity, namely water, ethyl

acetate, and n-hexane by looking at the microscopic effect of the density of collagen formed. Collagen density examination was chosen because collagen plays an important role in the wound healing process, among others, the role in hemostasis, interaction with platelets, interaction with fibronectin, increasing cellular components, increasing growth factors, and promoting fibroplasias and epidermal proliferation. However, the formation of collagen tissue in the DM condition becomes obstructed which causes wound to be difficult to heal [9-11]. Therefore, based on this, in the present study, we examine the effect of extract fraction of *Mm* (Lour.) on diabetic wound healing by evaluating the collagen density.

MATERIALS AND METHODS

Chemicals and reagents

Chemicals i.e. ethanol, n-hexane, ethyl acetate, chloroform, ether, and reagents i.e. gallic acid and xylol were purchased from Merck [Indonesia]. Other chemicals such as ketamine HCl and xylazine were purchased from Guardian Pharamatama [Indonesia] and Interchemie Werken [imported from Holland]. From Sigma-Aldrich [Indonesia], Trichrome Stains, citrate buffer solution and Streptozotocin (STZ) were purchased. All other chemicals and reagents used for the analysis were analytical grade. Distilled water (aquadest) was used throughout the investigation.

Animal model

This study used 25 male albino wistar rats in early adulthood weighing between 150-200 g and each was kept in an individual cage with a standard feed of ad libitum food and water. The rats were obtained from CV. Gamma Scientific Biolab, Malang, Indonesia. They were divided into 5 groups with 5 rats in each group (n=5), i.e.,

K+(positive control group with gentamicin administration), K- (negative control group with aquadest administration), K1 (ethyl acetate fraction), K2 (water fraction), and K3 (n-hexane fraction). The rats were adapted for 7 d, then induced into DM by injecting STZ in citrate buffer solution intraperitoneal each at 40 mg/kg body weight (kgBW) dose and was given access to drink 10% dextrose for 24 h [12]. Rats with non-fasting blood glucoses less than 200 mg/dL on day 5 after STZ induction were excluded. Glucose examination was done once a week to ensure the diabetic condition.

Wound excision

Diabetic wounds were made by Morton excision techniques [13]. Rats were given a combination of ketamine at 50 mg/KgBW dose and xylazine at 10 ml/KgBW dose intramuscularly. Particular skin area was shaved and the skin of impressed area was excised to the full-thickness to obtain a rectangle wound area of about 25x25 mm. Measurement of the wound area to determine the percentage of wound healing [14] and administration of 25 mg dose of each fractions were carried out every other day once a day after the wound was given.

This study followed the standard of ethics of Health Law research number 23/1992 and has obtained ethical approval number 1175/H25.1.11/KE/2017 from the Medical Faculty, University of Jember. The type of research used in this research was *in vivo* true experimental laboratories with the research design used was post-test only control group design.

Formulation of the fraction of *Mm* (Lour.) extract

The plant material, *Mm* (Lour.), was collected July 2017 from Klaten, Central Java Province, Indonesia. It is identified and deposited by

Herbarium Jemberiense, Biology Department, Mathematic and Natural Science Faculty, University of Jember (84/HB/7/2017). The viscous ethanol extract of *Mm* (Lour.) was fractionated with three different polarity types of solvents, i.e. water, ethyl acetate and n-hexane [8]. 50 grams of the viscous extract was added to 100 ml of water and stirred until homogeneous. This water fraction was separated subsequently in a successive partition using n-hexane and ethyl acetate with a ratio of 2:3, repeated three times. The partition was evaporated with rotavapor to get n-hexane and ethyl acetate fraction. The water fraction was concentrated with freeze dryer until viscous fraction was obtained.

Histopathology parameter

The observation of collagen density was carried out on day 11 (n=5 per group) by taking pictures of histopathological preparations of Trichome Masson's staining with Olympus DP21 series microscope with 400x magnification each in 6 visual fields then processed by using image software (data represented in percentage as mean±SEM). Statistical tests were performed with the One Way Anova test or Kruskal Wallis test and were continued with Post Hoc Tukey or Mann-Withney [15, 16].

RESULTS

Based on fig. 1(A) and 2, there was an increase in the wound healing percentage, the largest percentage of healing occurred in K2 (the group of water fractions), followed by K+(gentamicin group). The lowest percentage of wound healing occurred in K-(aquadest group). Percentage difference of wound healing occurred from day 1 to day 11. The highest percentage of wound healing on day 1 was the K3 (33.73±4.4%) and on day 3 was K+(50.46±6.3%). The top percentage of healing on day 5, 7, 9, and 11 were K2 with the percentage of 68.73±2.7%, 81.31±1.8%, 90.08±2.3% and 93.4±1.3%.

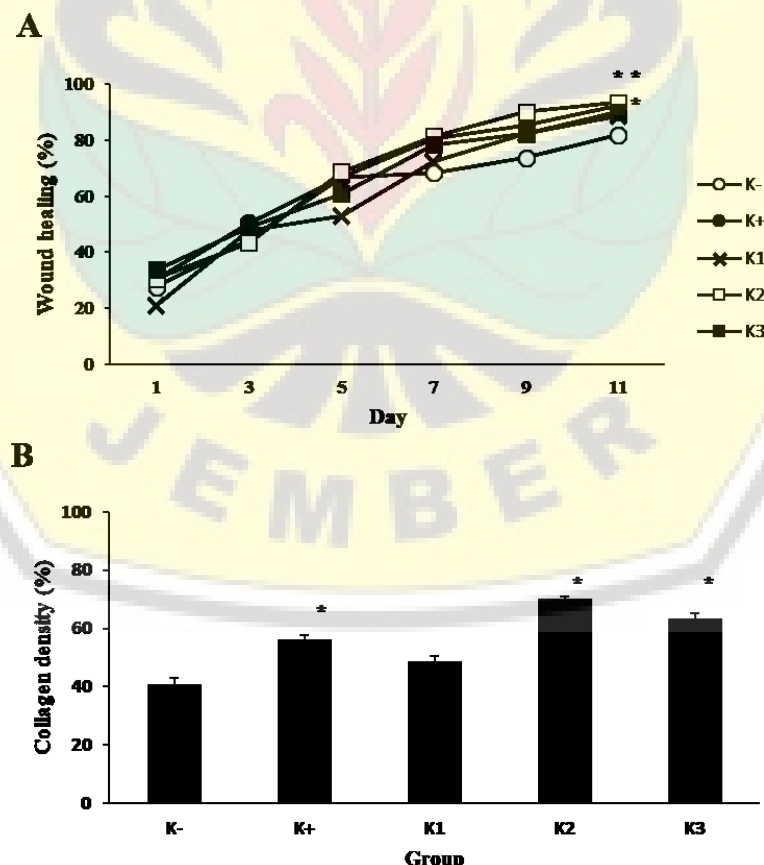


Fig. 1: The evaluation of diabetic wound healing by parameter of, (A): Wound healing percentage, (B): Collagen density, data represented in percentage as mean±SEM (n=5), *significant value at P<0.05 compared aquadest (K-: negative control group with aquadest administration, K+: positive control group with gentamicin administration, K1: ethyl acetate fraction, K2: water fraction, K3: n-hexane fraction)

The results of the wound healing percentage analysis were not significant on days 1, 3, 5 (One Way Anova test) and 7, 9 (Kruskal Wallis test). However, on the day 11, there were significant results ($p < 0.05$; One Way Anova test) in the negative group compared with the positive groups, water fraction, and n-hexane fraction ($p = 0.008$; $p = 0.003$; $p = 0.014$; Post Hoc Turkey test), with each $n = 5$. Then followed by using post hoc, there was also no significant ($p > 0.05$) or there was no meaningful difference in the group of fractions when compared with the positive groups.

The order of collagen density average measured with ImageJ software (fig. 1(B)) from low to high was K- ($40.59 \pm 2.4\%$), K1 ($48.21 \pm 2.4\%$), K+ ($55.94 \pm 1.7\%$), K3 ($63.01 \pm 2.1\%$) and K2 ($70.02 \pm 1.0\%$). The collagen density analysis showed significant results ($p = 0.000$) when compared among groups by One Way Anova test. Tukey's post hoc test showed significant results ($p = 0.000$; $p = 0.000$) of the group given gentamicin, water fraction,

and n-hexane fraction compared to the group given aquadest, with each $n = 5$. The group given the water fraction had no significant difference ($p = 0.989$) to the group that was given gentamicin. The water fraction group also showed no significant difference ($p = 0.949$) to the n-hexane fraction group. While the group given the ethyl acetate fraction showed a non-significant difference ($p = 0.095$) to the group given aquadest.

Fig. 3 shows collagen deposit (distinguished from others by blue color) observed in Masson's trichome staining. The collagen density was very low in the control group and ethyl acetate fraction group but the gentamicin group as control positive and other treated groups showed moderate and high collagen density. Loose collagenous matrix was found in the control group and ethyl acetate fraction group whereas in gentamicin group and other treated groups more compact and matured collagen deposit was observed. This result was similar to Kirubandanan *et al.* research observation of collagen deposit on day 12 of healing [17].



Fig. 2: Wound area on day 11 before termination, (A) Control; (B) Gentamicin; (C) Ethyl acetate fraction; (D) Water fraction; (E) n-hexane fraction

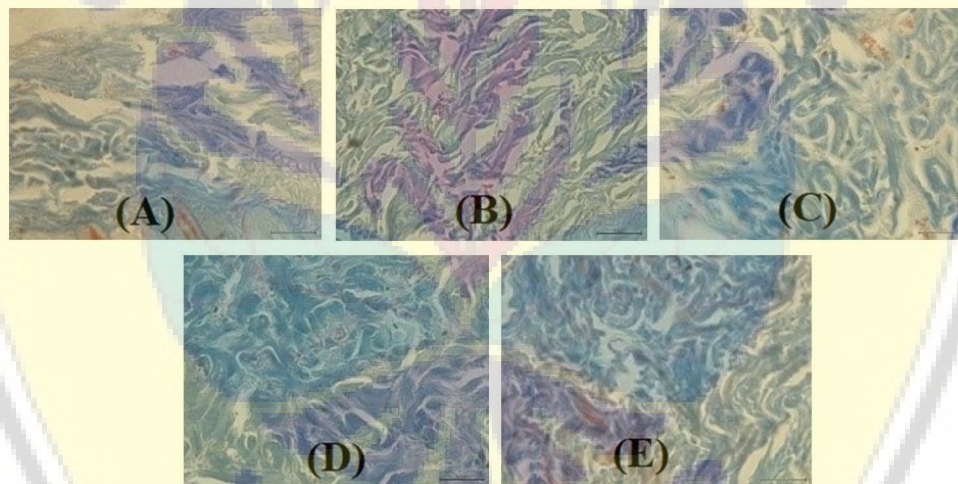


Fig. 3: Photomicrographs of rat skin tissues cross-section with Masson's trichome staining (collagen was distinguished from others by blue color) in 400x magnification, (A) Control; (B) Gentamicin; (C) Ethyl acetate fraction; (D) Water fraction; (E) n-Hexane Fraction

DISCUSSION

There were significant results in the percentage of day 11 wound healing and collagen density (fig. 1, 2 and 3). This showed that there was an effect of fractionation of *Mm* (Lour.) on the healing of diabetic wounds both macroscopically (wound area) and microscopic (collagen density). The administration of aquadest in the negative group showed the lowest percentage of wound healing and collagen density. This may occur because aquadest does not contain any other substances than H_2O . Therefore, it only functioned to clean wounds from foreign objects attached to the wound [18]. Gentamicin administration had lower effect on collagen density than the water fraction and n-hexane fraction. This may occur because gentamicin contains the active ingredient of Gentamicin sulfate which was intended as an antibacterial. Whereas the pathophysiology of the occurrence of diabetic wounds is not only due to the occurrence of infection but also due to complications of neuropathy and blood vessel abnormalities in patients with DM. These complications cause disturbances in immune cell function,

ineffective inflammatory response, endothelial cell dysfunction, neovascularization disorders, decreased collagen synthesis, worsening of epithelialization, decreased angiogenesis process and the inability of fibroblasts to form a maximum extracellular matrix [19].

This study showed that water fraction obtained the highest percentage of wound healing and collagen density compared to other treatment groups. There was a significant difference between the administration of water fraction and the administration of gentamicin or ethyl acetate fraction. This may occur because the water reaction has a higher antioxidant activity than the ethyl and n-hexane fractions. The water fraction uses a polar solvent that can dissolve the flavonoids which are glycosidic and polyphenol groups and tannin substances which are higher than other fractions. Hence, due to the reason above, the administration of water fraction showed more optimal results in wound healing [20, 21]. The use of water as a solvent has dissolved vitamin C. Therefore, it contained vitamin C while the other fractions did not. Vitamin C is one of the enzyme cofactors in the process of forming procollagen in the

lysine and proline hydroxy stages. The protocol will become procollagen. Procollagen will become tropocollagen. Tropocollagen will divide into filaments and filament will become fibril. Fibrils will join to form fibers or collagen fibers. This was one of the causes that collagen density in the water fraction group showed a higher density than the other groups.

The result also showed that n-hexane fraction collagen density was lower than the water fraction group. This may occur because the content of antioxidants and flavonoids in this group was less than in the type of solvent at other polarity levels [22, 23]. The group administered with ethyl acetate showed the lowest percentage of wound healing and collagen density among the fraction groups even when compared to the group administered with aquadest, it showed a non-significant difference. This is probably because ethyl acetate fraction has more toxic properties than the n-hexane and water fractions. Therefore, its pharmacological effects of wound healing were lower than the toxic effects produced [24, 25].

Although this study covered different groups of solvents, it did not include variations in doses of the fraction. Therefore, it did not measure the effective doses. In addition, this did not use carrier material as a mixture of the fractions. Therefore, further research shall include varying dosages to determine the effective dose and toxicity of the *Mm* (Lour.) and use carrier material as a mixture of fractions to make therapy to be more effective.

CONCLUSION

Based on the results obtained, it can be concluded that some fractionation of *Mm* (Lour.) extract had an effect on diabetic wound healing. Water fraction and n-hexane enhanced healing. Meanwhile, the ethyl acetate fraction showed no effect in the healing process. The most effective fraction, water fraction, is potential to be developed further as a topical drug.

ACKNOWLEDGMENT

This paper is based upon research supported by Ministry of Research, Technology and Higher Education of The Republic of Indonesia under Applied Product Research Funding.

AUTHORS CONTRIBUTIONS

All the authors have contributed equally

CONFLICTS OF INTERESTS

All authors have none to declare

REFERENCES

- International Diabetes Federation. IDF diabetes atlas. 6th ed. IDF; Brussels, Belgium; 2013. Available from: www.idf.org/diabetesatlas. [Last accessed on 20 Aug 2018].
- Ministry of Health of the Republic of Indonesia. Situation and analysis of diabetes. Data and information center of Ministry of Health of the Republic of Indonesia, Indonesia; 2014.
- Chin LCH, Boulton AJM. The diabetic foot: epidemiology, risk factor, and standards of care in general surgery. Springer; 2009. p. 1867-76.
- Nurlitasari N. Wound healing effect of perorally administration of combination sirih merah (*Piper cf. fragile*, Benth) leaves and pegagan (*Centella asiatica*, (L.) Urb) herbs infusions in the diabetic rat. Unpublished script. Universitas Indonesia, Depok, Indonesia; 2015.
- Hidayat FK, Elfiah U, Sofiana KD. Comparison of the number of macrophage in full thickness wound incision between *Merremia mammosa* extract treatment and NaCl in male wistar rats. J Agromed Med Sci 2015;1:9-13.
- Sofiana KD, Elfiah U, Ulfa EU. The effect of *Merremia mammosa* Lour. on wound healing of hyperglycemic male wistar rats. Unpublished articles. Fakultas Kedokteran Universitas Jember, Jember, Indonesia; 2015. Available from: <http://repository.unej.ac.id> [Last accessed on 20 Aug 2018].
- Julianto IGP. Effect of *Merremia mammosa* (lour) on wound healing process and blood sugar levels in hyperglycemic male wistar rats. Unpublished script. Fakultas Kedokteran Universitas Jember, Jember, Indonesia; 2015.
- Mukhriani T. Extraction, separation of compounds and identification of active compounds. J Kesehatan 2014;7:361-7.
- Novriansyah R. The difference of collagen density around wistar mice wound incision dressing with conventional gauze and occlusive hydrocolloid for 2 and 14 d. Unpublished thesis. Universitas Diponegoro, Semarang, Indonesia; 2008.
- Dewi AS. Antioxidant test of ethyl acetate fraction and water fraction of green tea ethanol extract through hydroxyl radical examination with deoxyribose method. Unpublished script. Universitas Sanata Dharma, Yogyakarta, Indonesia; 2007.
- Sugara TH, Irawadi TT, Suprpto HH, Hanafi M. Anti-bacteria activity of ethyl acetate fraction of bandotan leaves (*Agerantum conyzoides* L.). Jurnal Ilmiah Ibnu Sina 2016;1:88-96.
- Damasceno DC, Netto AO, Iessi IL, Gallego FQ, Corvino SB, Dallaqua B, et al. Streptozotocin-induced diabetes models: pathophysiological mechanisms and fetal outcomes. Hindawi Publishing Corporation. BioMed Res Int 2014;1-11. <http://dx.doi.org/10.1155/2014/819065>
- Morton JJ, Malone MH. Evaluation of vulnerary activity by an open wound procedure in rats. Arch Int Pharmacodyn Ther 1972;196:117-26.
- Wathon N, Hasanah AN, Mohammed AFA, Pratiwi ED, Mahmudah R. Accelerated wound healing ability of sacran hydrogel film by keratinocyte growth factor in alloxan-induced diabetic mice. Int J Appl Pharm 2018;10:57-61.
- Badr G. Camel whey protein enhances diabetic wound healing in a streptozotocin-induced diabetic mouse model: the critical role of β -defensin-1,-2 and-3. Lipids Health Disease 2013;12:46.
- Chen Y, Yu Q, Xu C. A convenient method for quantifying collagen fibers in atherosclerotic lesions by imagej software. Int J Clin Exp Med 2017;10:14904-10.
- Kirubandanan S, Bharathi R, Renganathan S. Histological and biochemical evaluation of wound regeneration potential of *Terminalia chebula* fruits. Asian J Pharm Clin Res 2016;9:228-33.
- Amaliya S, Soemantri B, Utami YW. Effects of pegagan leaves (*Centella asiatica*) extract in accelerating contaminated wound on *Rattus norvegicus* wistar strain. Jurnal Ilmu Keperawatan 2013;1:19-25.
- Waspadji S. Kaki Diabetes. Editor Setiati S, Alwi I, Sudoyo AW, MSK, B Setiyohadi, Syam AF. Buku Ajar Ilmu Penyakit Dalam. (Book of Internal Medicine). Edisi Empat. Jilid 2. Jakarta: Interna Publishing; 2015.
- Prahesti NR, Suzery M, Cahyono B. The antioxidant activities, phenolic total, and cytotoxicity of extract and fractions of *Aloe Vera* Linn). Jurnal Sains Dan Matematika 2015;23:50-4.
- Rahayu S, Kurniasih N, Amalia V. Ekstraksi dan identifikasi senyawa flavonoid dari limbah kulit bawang merah sebagai antioksidan alami. Al Kimiya 2015;2:1-8.
- Pratiwi L, Fudholi A, Martien R, Pramono S. Ethanol extract, ethyl acetate extract, ethyl acetate fraction, and n-hexane fraction mangosteen peels (*Garcinia mangostana* L.) as the source of bioactive substance free-radical scavengers. J Pharm Sci Clin Res 2016;1:71-82.
- Faisal AP, Azhari A. Identification of secondary metabolites and antioxidant activity of mojo leaves (*Aegle Marmelos* L.) against DPPH (1,1 Difenil-2-pikrilhidrazil) free radicals. Mahakam Med Laboratory Technol J 2017;11:27-36.
- Pasilala FB, Daniel C, Saleh C. Toxicity test (brine shrimp lethality test) and antioxidant activity of sintrong leaves (*Crassocephalum Crepidioides*) 2,2-diphenyl-1-picrylhidrazil (DPPH) method. Jurnal Kimia Mulawarman 2016;14:13-4.
- Tanaya V, Retnowati R, Suratmo S. Fraksi semi polar dari daun mangga kasturi (Semi polar fractions of *Mangifera casturi Kosterm*). Kimia Student J 2015;1:778-84.