



Evaluation of Wound Healing Properties of *Dryopteris filix-mas* Leaf and Root Extracts on Albino Rats

Earnest Oghenesuvwe Erhirhie^{a,b,*}, Cathrine Odinakachi Anyim^a, Ugochukwu Moses Okezie^c, Sabastine Obiora Igboeme^a, Emmanuel Emeka Ilodigwe^a

^aDepartment of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria, ^bDepartment of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Chukwuemeka Odumegwu Ojukwu University, Igbariam, Nigeria, ^cDepartment of Pharmaceutical Biology and Biotechnology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria.

Abstract

Dryopteris filix-mas (*D. filix-mas*), belonging to the family of *Dryopteridaceae* is a swampy fern that is popularly used by the Southern Nigerian dwellers in the treatment of wounds, hemorrhages, boil and other diseases. In order to authenticate its folkloric benefits in wounds, this study evaluated its wound healing activity using excision model. A total of fifty (50) Wistar rats were randomized into ten groups of five animals each. After creation of surface wounds, group 1 received paraffin base (control). Group 2 received Povidon iodine (standard). Groups 3, 4, 5 and 6 received 1.25, 2.5, 5 and 10% (w/w) of an ethanol leaf extract of *D. filix-mas* formulated with paraffin base respectively. Groups 7, 8, 9, 10 were treated with 1.25, 2.5, 5 and 10% (w/w) of ethanol root extract *D. filix-mas* formulated with paraffin base respectively. Treatments were topically applied to wounds once daily and healing rate was monitored every 3 days for 21 days. Wound swaps were taken on day 10th and day 20th for bacteria load determination. *In-vitro* antimicrobial activities of the leaf and root extract were tested against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* using agar-well diffusion method. Higher concentrations (5 and 10%) of the leaf and root extracts exhibited better wound healing activities more than lower concentrations. The leaf extract produced a better healing rate (wound contraction), antimicrobial activity and body weight regaining activities more than the root extract. This study validates the traditional use of *D. filix-mas* in the treatment of wounds.

Keywords: Antimicrobial activity, *Dryopteris filix-mas*, Leaf extract, Root extract, Wound healing, Wistar rats.

Corresponding Authors: Erhirhie Earnest Oghenesuvwe, Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria, Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Chukwuemeka Odumegwu Ojukwu University, Igbariam, Nigeria.

Tel: +234-7060434974

Email: erhirhieochuko@yahoo.com.

Cite this article as: Erhirhie E. O., Anyim C. O., Okezie U. M., Igboeme S. O., Ilodigwe E. E., Evaluation of Wound Healing Properties of *Dryopteris filix-mas* Leaf and Root Extracts on Albino Rats, 2021, 17 (1): 79-90.

1. Introduction

Wounds are physical injuries to any part of the body. They may result from fire accident, chemicals, radiation, electricity or surgery [1]. Wounds involve laceration or a break of the skin that causes distortion in the normal skin structure and function [2]. Complicated wounds, including burns, chronic wound

ulcers, and orthopedic wounds play a significant part in morbidity and mortality. Statistics revealed that about 34% of diseases such as boils, diabetes, chicken pox, tumors, athlete's foot, sporotrichosis, and necrotizing ascitis that occur worldwide are associated with the wound pathogenesis [3-5]. A study carried out by Adigun and co-workers in a tertiary hospital in Kwara State, Nigeria revealed that 31.55% of total hospital patients suffered from various wounds and the cost spent on wound care was quite burdensome [6].

Several synthetic drugs such as povidone iodine, methoxamine, silver sulfadiazine, polyhexamethylene biguanide are useful agents in the treatment of wound [7]. However, some limitations such as high cost, allergic reactions, bacterial resistance are associated with the use of these conventional agents in the treatment of wounds [7]. Thus, efforts have been made to discover alternative approaches in the treatment and management of various wounds.

Among alternative remedies, medicinal plants have played significant role in the treatment of wounds since prehistoric times [8]. Plant extracts have been used as poultices for the purpose of stopping hemorrhage and promoting wound healing [9]. They are effective, accessible, affordable, and have no or less side effects than some conventional wound healing drugs [10]. For example, *Hibiscus sabdariffa*, *Aloe vera*, honey, *ageratum conyzoides*, *Azadirachta indica*, *Hyptis suaveolans*, *Moringa oleifera* and other medicinal plants have been validated and

documented to have wound healing properties based on their folkloric information [2, 5, 8, 11].

D. filix-mas (Linn.) Schott), also known as male fern, wild fern, bear's paw is a medicinal plant used traditionally in the treatment of wound [12]. It is a strong ornamental fern that is commonly found in damp, shady and swampy environments. It is widely distributed in various parts of North and South America, Europe, Asia, United States of America and Africa including Nigeria [13]. *D. filix-mas* is an evergreen plant that reaches a maximum height of 1.5 m, with a single crown on each rootstock. The leaves consist of 20-35 pinnae on each side of the rachis. The leaves taper at both ends, with the basal pinnae about half the length of the middle pinnae [14]. It is the male version of the widespread lady fern, *Athyrium filix-femina*, which is considered as a traditional vegetable [15]. Its decoction is applied topically in the treatment of mumps, carbuncles, abscesses, boils and sores provoked by severe burns. An infusion of the rhizome is applied topically in rheumatism, septic wounds, hemorrhoids and ulcers in the Republic of Azerbaijan [16]. It is used among rural dwellers in Lebanon in the treatment of neuralgia and rheumatic disorders [17].

In Southern parts of Nigeria the root and leaf decoctions are used topically for wounds and various hemorrhage, such as epistaxis, menorrhagia and postpartum hemorrhage, inflammation, rheumatoid arthritis and worm infestations. Recent studies have reported its antidiarrheal [18], tocolytics [19], teratogenic [20], and anti-inflammatory [21] effects.

Although, it is a popularly known for its folkloric wound healing potential, there is yet to be scientifically proven research to validate this ethno medicinal claim. Therefore, we undertook this study to evaluate the wound healing effects of *D. filix-mas* using excision model in Wistar rats.

2. Materials and Methods

2.1. Animals

Albino rats of average weight 139.9 ± 5.38 g, procured from the animal facility of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka were used for the study. They were acclimatized for a period of one week with free access to rodent feed (Grower's feed) and tap water *ad libitum*. They were taken care of in conformity with the National Institute of Health Guidelines for the care and use of laboratory animals for research purpose (Pub No. 85-23, revised 1985).

2.2. Materials

Water bath, weighing balance, incubator, measuring cylinder, dissecting set, muslin cloth, spatula, forceps, permanent markers, meter rule, petri dishes, cotton wool, ethanol, paraffin, povidone iodine, lignocaine + adrenaline (ADRELID[®]), chloroform, muller Hinton Agar (MHA), hair removal cream (Veet).

2.3. Plant Collection, Authentication and Extraction

Fresh leaves and roots of *D. filix-mas* were collected during the month of March from a swampy location beside a botanical

garden in Amawbia, Anaocha Local Government Area, Anambra State, Nigeria. The plant specimen (with voucher number, UBHd285A) was authenticated by Dr. Akinnigbosun H. I of the Department of Botany, Faculty of Life Sciences, University of Benin, Edo state.

Fresh leaves and roots of *D. filix-mas* were washed and cut into smaller pieces, air-dried at room temperature for seven days. Crisply dried leaves and roots were pulverized, using a grinding machine. Afterwards, 450 g of the powdered leaves and 550 g of the powdered roots were macerated in 2250 ml and 2750 ml 80% ethanol respectively in the ratio of 1:5. The mixtures were agitated continually for 72 hours and were filtered using a muslin cloth. The entire filtrate recovered was concentrated to a paste-like form using a water bath set at a temperature of 45⁰C. A greenish paste-like leaf extract weighing 77.14 g (17.14%w/w) and a light green root extract weighing 93.88 g (17.07%w/w) were recovered after the extraction [20].

2.4. Phytochemical Screening

A qualitative phytochemical test was carried out by adopting the methods of Sofowora, Evans and Trease, and Harborne as described by Yadav and Agarwala [22].

2.5. Ointment Preparation

Simple ointments of ethanol leaf and root extracts of *D. filix-mas* were prepared by fusion method using simple paraffin base. Four concentrations, 1.25%, 2.5%, 5% and 10% of the root and leaf extracts were prepared using an ointment base.

2.6. Test Microorganism

Staphylococcus aureus, *Pseudomonas aeruginosa* and *Escherichia coli* strains implicated in wound infection were gotten from Pharmaceutical Microbiology and Biotechnology Laboratory, Nnamdi Azikiwe University, Agulu, Nigeria. These isolates were reconfirmed by subjecting them to gram staining as well as specific biochemical test.

2.7. Preparation of Media

Manitol salt agar (111 g/L) and MacConkey agar (48.5 g/L) were prepared following the manufacturer's specification by dispersing the required quantity of agar in distilled water. It was homogenized and then sterilized by autoclave at 121°C for 15 minutes.

2.8. Determination of Antimicrobial Activity (Agar-well diffusion assay) on Root and Leaf Extracts

The antibacterial activity of the extracts was evaluated by cup plate agar diffusion method described by Okezie et al [23]. The microorganisms used in this test were from the human pathogenic bacteria, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. The bacterial cultures were adjusted to 0.5 McFarland turbidity standards. Thereafter, each of the test organisms was seeded onto sterile Mueller-Hinton Agar MHA (Oxoid, Difco USA) and Sabouraud dextrose agar plates using sterile swab, (diameter: 90

mm). A sterile cork borer was used to make wells (6 mm in diameter) on each MHA. Stock concentrations of the extracts were made using sterile distilled water as diluent. Here, 800 mg of each of the extract was dissolved in 2 ml of sterile distilled water. Thereafter, serial dilutions were made using the same diluents to get graded concentrations (400, 200, 100, 50, 25, and 12.5 mg/ml). Aliquot (80 µl) of each concentration was applied to each well previously seeded with the test organism. The cultures were incubated at 37°C for 24 hrs. Antimicrobial activity was determined by measuring the zone of inhibition (ZOI) around each well (excluding the diameter of the well). For each concentration, replicate trials were conducted against test organisms.

2.9. Wound Healing Studies

Evaluation of wound healing activity of the root and leaf extracts was carried out using wound excision model in Wister albino rats, according to the method described by Vinay *et al.* [24]. Prior to the creation of the wound, hair on the mid-back of animals was carefully shaved using a pair of scissors and tiny hair remaining were completely removed using hair removal cream (Veet). Thereafter, animals were anesthetized by injection of 0.3 ml of a local anesthetic (Lignocaine + Adrenaline) into the shaved portion. Circular wounds of approximately 1.5 to 2.0 cm diameter were created along a circumference that was outlined with the orifice of a test tube stained with methylene blue. The wounds were left undressed for 24 hours.

Thereafter, animals were divided into ten groups of five animals each. Animals in group 1 were treated with paraffin base and they served as the control. Animals in group 2, which served as standard received Povidon iodine. Groups 3, 4, 5 and 6 were treated with 1.25, 2.5, 5 and 10% (w/w) of ethanol leaf extract-ointment of *D. filix-mas* respectively. Animals in groups 7, 8, 9, 10 were treated with 1.25, 2.5, 5 and 10% (w/w) of ethanol root extract-ointment of *D. filix-mas* respectively. Treatments were topically applied once daily and wound closure rate was assessed every 3 days by measuring the longitudinal and transverse diameter of the wound, using a pair of dividers. Bacterial load was assessed on day 10th and day 20th, while healing rate, body weight gain and inhibition in wound bacteria count were calculated using the formulae below.

Healing rate =

$$\frac{\text{Wound diameter at the baseline} - \text{Wound diameter at any day}}{\text{Wound diameter at baseline}} \times \frac{100}{1}$$

Body weight gains =

$$\frac{\text{Final body weight on day 20th} - \text{Initial body weight before treatment}}{\text{Final body weight on day 20th}} \times \frac{100}{1}$$

Reduction in wound bacteria count =

$$\frac{\text{Bacteria count on day 10th} - \text{Bacteria count day 20th}}{\text{BactBacteria count on day 10th}} \times \frac{100}{1}$$

2.10. Statistical Analyses

Data were presented as Mean \pm standard error of mean (SEM), n = 5 and were analyzed by one way analysis of variance (ANOVA) using SPSS version 20. Differences between

mean were considered statistically significant at p<0.05.

3. Results and Discussion

3.1. Phytochemical Composition

Reducing sugar, proteins, were present in similar proportion in both extracts. Tannins, flavonoids and alkaloids were more abundant in the leaf extract. Anthraquinones were absent in both extracts while saponins and steroids were absent in the root extract ([Table 1](#)). More phytoconstituents, saponins, alkaloids, sterols, terpenoids, glycosides, tannins, flavonoids, reducing sugar and proteins were present in the leaf extract than the root extract, which is an indication that the leaf extract may retain more healing potentials against wounds. Jian *et al*, [25] reported that saponins and flavonoids show evidence of wound healing activity. Terpenoids, due to their acerbic and antimicrobial properties have been reported to promote wound contraction and increased rate of epithelialization [26]. Total phenolic contents and antioxidant activity of the leaf extract previously reported by Sekender *et al* [14] also supports the healing properties exhibited by the leaf extract. Suppression of inflammatory process, oxidative stress processes occurring in wounds have been attributed to polyphenols, which have potentials to terminate chain reactions associated with oxidative damage in biological system [5]. Polyphenols have also been reported to prevent edema and itching associated with wounds via inhibition of allergic mediators such as histamine and

serotonin [27, 28]. To this end, we hypothesize that flavonoid, quercetrin which was an abundant phyto-compound responsible for the anti-inflammatory activity of the leaf extract in our earlier study [21] could be strongly connected with the wound healing activity of *D. filix mas*.

3.2. Antimicrobial Activity

The root extract did not show activity against *E. coli* and *P. aeruginosa*, except little activity against *S. aureus*. On the other hand, the leaf extract produced better activity against *S. aureus* when compared to the root extract. Also, the leaf extract inhibited the growth of *P. aeruginosa* at higher concentrations, 200 and 400 mg/ml with minimum inhibitory concentrations of 200 and 50 mg/mL for *P. aeruginosa* and *S. aureus* respectively ([Table 2](#)). Microorganism, *P. aeruginosa* and *S. aureus* are implicated in the pathogenesis of wound. In this study, we found that the leaf extract of *D. filix-mas* showed a concentration dependent inhibition against *P. aeruginosa* and *S. aureus* more than the root extract which only showed less inhibition against *S. aureus*. This inhibitory activity of the leaf extracts could have contributed to its fast healing rate by minimizing super infections that are implicated in wounds. This is supported by the antimicrobial activity of methanol leaf extract of *D- filix-mas* against the growth of *E. coli*, *S. aureus*, *P. aeruginosa* as well as *S. abony* and *E. faecalis* [29]. Some polyphenols have been shown to reduce the growth of microorganisms such as *S. aureus* and *E. coli*, which are implicated in wound progression [30].

3.3. Wound healing Effect

There was a time dependent healing rate in group 1 treated with soft paraffin (negative control), Povidone iodine (positive control) and various concentrations of plant extract. An exception to these were reduced healing rates observed on day 3 in control, 2.5% leaf extract, 1.25 and 2.5% root extract treated groups ([Table 3](#)).

Progressive healing rate observed in this study suggests that healing is a gradual and continuous process which involves several phases such as hemostasis, inflammation, leukocyte migration, proliferation and maturation as substantiated by the study of Boateng *et al* [1] who reported that wound healing is a progresses process. Faster rate of wound closure and contraction following topical application of higher concentrations, 5 and 10% of ethanol leaf and root extracts of *D. filix-mas* suggests that substantial healing effect could be attained at higher concentration. The healing effect could be attributed to flavonoids, and other phytoconstituents in the extracts.

There was reduced healing rate in groups treated with Povidone iodine, 1.25 and 2.5% leaf extract as well as 1.25 and 2.5% root extract when compared to healing rate in the negative control group on various days. Although, healing rate in Povidone iodine group was slightly higher than 1.25 and 2.5% in leaf extract as well as 1.25 and 2.5% in root extract treated groups on day 21st ([Table 3](#)). There was time dependent closure in wounds treated with the negative control (soft paraffin) and positive control (povidone iodine).

Healing rates in 5.0 and 10.0% leaf and root extracts were higher than healing rates in both negative and positive control groups as well as 1.25 and 2.5% treated groups. This was consistent from day 3 through day 21 (Table 3).

Wound healing is a natural process which takes place by regeneration of dermal and epidermal tissue. When tissue damages are not treated and properly managed, it could lead to chronic inflammation and secondary infections, thus causing more damage to surrounding tissues [10]. Whenever there is an injury, a set of overlapping procedures take place in a predictable manner to restore the damage [25]. Effective remedies in the treatment of wounds have potentials of minimizing secondary infections, inflammation and promotion of tissue regeneration [10].

3.3.1 Effects of Treatments on Wound Microbial Load

There was no reduction in *Staph aureus* count of wounds treated with soft paraffin and Povidone iodine between day 10th and day 20th. However, there were reductions in *Staph aureus* counts in wound treated with various concentrations of *D. filix-mas* leaf and root extracts (Table 4). Reduction in *S. aureus* count (42.42, 45.26, 52.57 and 88.59%) was dose dependent in the leaf extract treated groups, but the reduction (89.27, 78.30, 87.60 and 85.09%) was not dose dependent in the root extract treated groups (Table 4). *E. coli* was very minimal in wounds treated with soft paraffin (control group) on day 20th (100.00%

reduction in bacteria count) when compared to other groups. This may be attributed to the ability of *E. coli* to have degraded the extract thereby allowing more proliferation. On the other hand, paraffin alone, being an organic substance may not have been easily degraded by *E. coli*. There was a dose dependent reduction rate of *E. coli* in wounds treated with 1.25, 2.5, 5.0 and 10.0% of leaf extract. *E. coli* count in wounds treated with 1.25, 2.5, 5.0 and 10.0% of root extract was reduced on day 20th, but was not dose dependent (Table 4). Groups treated with povidone iodine and soft paraffin showed no reduction in *E. coli* when compared to a concentration dependent reduction in the group treated with different concentrations of leaf extract of *D. filix-mas*. This suggests that povidone iodine and soft paraffin wax do not possess antimicrobial properties against *E. coli* associated wound infections.

3.3.2. Effects on Body Weight

Higher body weight gain was observed in animals treated with povidone iodine, 1.25, 2.5, 5.0 and 10.0% leaf and root extracts of *D. filix-mas* when compared to body weight gain of control group (5.83%). Body weight gain of animals in the leaf extract treated groups was higher than body weight gain of animals in root extract treated groups, although body weight was not dose dependent (Table 5). Higher weight gain observed in the leaf extract groups could be as a result of its wound healing effect and presence of phytoconstituents which could promote feed intake and weight gain (Erhirhie and Ilodigwe, 2019).

4. Conclusion

This study showed that 5 and 10% ethanol leaf extract-ointments of *D. filix-mas* facilitated wound healing more than root extract and standard drug, povidone iodine. Possible mechanisms include antioxidant, antimicrobial and anti-inflammatory processes which could be attributed to flavonoids, the most abundant phytochemicals in the leaf extract. From the foregoing, high concentrations of *D. filix-mas* could be effective in the treatment of surface wounds. This study therefore validates the folkloric use of *D. filix-mas* in the treatment of wounds, boils and hemorrhages.

Acknowledgments

We are thankful to Dr. H.A. Akinnibosun who assisted with the plant specimen identification.

References

[1] Boateng JS, Kerr HM, Howard NES and Gillian ME. Wound Healing Dressings and Drug Delivery Systems: A Review. *J. Pharm. Sci.* (2008) 97:2892–923.

[2] Agyare C, Boakye YD, Bekoe EO, Hensel A, Dapaah SO and Appiah T. Review: African medicinal plants with wound healing properties. *J. Ethnopharmacol.* (2016) 177:85–100.

[3] Abbasi AM, Khan MA, Ahmad M, Zafar M, Jahan S and Sultana S. Ethnopharmacological application of medicinal plants to cure skin diseases and in folk cosmetics among the tribal communities of north-west Frontier province. *Pakistan J. Ethnopharmacol.* (2010) 128: 322–35.

[4] Solanki R and Nagori BP. A review on microorganisms causing wound infections on skin. *Asian J. Pharm. Tech.* (2013) 3(3): 119–22.

[5] Builders PF and Builders MI. Wound Care: Traditional African Medicine Approach. *Intech open* (2016) <http://dx.doi.org/10.5772/65521>. Accessed on June 5th, 2020.

[6] Adigun IA, Rahman GA, Yusuf IF and Ofoegbu CKF. The point prevalence and cost of wound management in a Nigerian teaching hospital. *Nig. Med. J.* (2010) 51(1): 23–25.

[7] Zhu JJ, Yao S, Guo X, Yue BS, Ma XY and Li J. Bioactivity-Guided Screening of Wound-Healing Active Constituents from American Cockroach (*Periplaneta americana*). *Molecules.* (2018) 23(1): 101.

[8] Wilson RW and Francis KW. Evaluation of Wound Healing Activity of Ethanolic Extract of Leaves of *Croton Megalocarpus* Using Excision Wound Model on Wister Albino Rats. *IJSR.* (2016) 4(3): 182-94.

[9] Piriz MA, Lima CAB, Jardim VMR, Mesquita MK, Souza ADZ and Heeck RM. Medicinal plants in the wound healing process: a literature review. *Rev. Bras. Plantas Med.* (2014) 16(3): 628-36.

[10] Marume A, Matope G, Katsande S and Khoza S. Wound Healing Properties of Selected Plants Used in Ethnoveterinary Medicine. 2017; 8(September), 1–10. <https://doi.org/10.3389/fphar.2017.00544>.

[11] Hadagali MD and Chua LS. The anti-inflammatory and wound healing properties of honey. *Eur. Food. Res. Tech.* (2014) 239(6): 1003–14.

[12] Nwosu MO. ‘Ethnobotanical studies on some pteridophytes of Southern Nigeria’, *Economic Botany* (2002) 56(3): 255–59.

[13] Duke JA. *Handbook of medicinal herbs*, Herbal Reference Library. 2nd ed. CRC Press, Florida USA (2001) 677.

[14] Sekendar AM, Mostafa K, Raihan MO, Rahman MK, Hossain MA and Alam MS. Antioxidant and cytotoxic activities of methanolic extract of *D. filix-mas* (L.) Schott leaves. *Int. J. Drug Dev. Res.* (2012) 4(2): 223-29.

[15] Mohammed ASM, Khan MMRL, Jabin SA, Abedin N, Islam MF and Shaha B. ‘Nutritional

quality and safety aspects of wild vegetables consume in Bangladesh. *Asian Pac. J. Trop. Biomed.* (2016) 6(2): 125–31.

[16] Batsatsashvili K, Mehdiyeva N, Kikvidze Z, Khutsishvili M, Sikharulidze S, Tchelidze D, Alizade V, Narel Y, Zambrana P and Rainer W. *Dryopteris filix – mas* (L) Schott Dryopteridaceae. Bussmann Springer International Publishing AG. R.W. Bussmann (ed.), *Ethnobotany of the Caucasus, European Ethnobotany*. (2016), DOI 10.1007/978-3-319-50009-6_87-1. Accessed on June 5th, 2020.

[17] Marc EB, Nelly A, Annick DD and Frederic D. Plants used as remedies antirheumatic and antineuralgic in the traditional medicine of Lebanon. *J. Ethnopharmacol.* (2008); 120: 315–34.

[18] Uwumarongie HO, Enike MA and Bafor EE. Pharmacognostic Evaluation and Gastrointestinal Activity of *Dryopteris filix-mas* (L.) Schott (Dryopteridaceae). *EJHCPR.* (2016); 2: 19 – 25.

[19] Bafor EE, Omokaro WO, Uwumarongie OH, Elvis-Offiah UB, Omoruyi O, Viegelmann CV and Edrada-Ebel R. *D. filix-mas* (Dryopteridaceae) leaves inhibit mouse uterine activity. *JOMPED.* (2017) 1(1):1-12.

[20] Erhirhie EO, Ezemokwe ON and Ilodigwe E. Teratogenic Effects of Ethanol Leaf Extract of *Dryopteris filix –mas* (L.) Schott. *AJNP.* (2018) 6(1): 573-83.

[21] Erhirhie EO, Emeghebo CN, Ilodigwe EE, Ajaghaku DL, Umeokoli BO, Eze PM, Ngwoke KG and Okoye FBC. *Dryopteris filix-mas* (L.) Schott ethanolic leaf extract and fractions exhibited profound anti-inflammatory activity. *Avicenna J. Phytomed.* (2019) 9(4): 396-409.

[22] Yadav RNS, Agarwala M. Phytochemical analysis of some medicinal plants. *J. Phytol.* (2011) 3(12): 10-14.

[23] Okezie UM, Eze PM, Okoye FBC, Ikegbunam MN, Ugwu MC and Esimone CO. Biologically active metabolites of an endophytic

fungus isolated from *Vernonia amygdalin*. *AJOPRED* (2017) 9(1): 24-26.

[24] Vinay K, Abdullah AK and Nagarajan K. Animal Models for the Evaluation of Wound Healing Activity. *IBDR* (2013): 3(5): 93-107. https://www.researchgate.net/publication/274010528_animal_models_for_the_evaluation_of_wound_healing_activity.

[25] Jian PS and Bari SB. Evaluation of wound healing effect of petroleum ether and methanolic extract of *Abemoschus manihot*, *Medikik malvaceae*, *Wrightia tinctoria* R. Br. Apocyanaceae in rats. *Braz. J. Pharmacogn.* (2010) 20:156-271.

[26] Umeh VN, Ilodigwe EE., Ajaghaku DL, Erhirhie EO, Moke GE and Akah PA. Wound-healing Activity of the Aqueous Leaf Extract and Fractions of *Ficus exasperata* (Moraceae) and its Safety Evaluation on Albino Rats. *J. Tradit. Complement. Med.* (2014) 1(4): 246-52.

[27] Juríková T, Mlček J, Sochor J and Hegedúsová A. Polyphenols and their Mechanism of Action in Allergic Immune Response. *Glob. J. Allergy.* (2015) 1(2): 37-39.

[28] Iba Y, Shibata A, Kato M and Masukawa T. Possible involvement of mast cells in collagen remodeling in the late phase of cutaneous wound healing in mice. *Int. Immunopharmacol.* (2014) 4:1873–80.

[29] Soare LC, Ferdes M, Stefanov S, Denkova Z, Nicolova R, Denev P, Bejan C and Paunescu A. Antioxidant activity, polyphenols content and antimicrobial activity of several native Pteridophytes of Romania. *Notulae Botanicae. Horti Agrobotanici Cluj Napoca.* (2012) 40(1): 53-57.

[30] Atawodi SE, Atawodi JC, Idakwo GA, Pfundstein B, Haubner R, Wurtele G, Bartsch H and Owen RW. Evaluation of the polyphenol content and antioxidant properties of methanol extracts of the leaves, stem, and root barks of *Moringa oleifera* Lam. *J. Med. Food.* (2010) 13(3): 710–16.

Tables:**Table 1.** Phytochemical constituents of the ethanol leaf and root extracts of *d. filix-mas*.

Phytochemical constituents	Leaf extract	Root extract
Tannins	++	+
Flavonoids	+++	+
Saponins	++	-
Steroids	++	-
Alkaloids	++	+
Terpenoids	++	++
Anthraquinones	-	-
Cardiac glycosides	+	++
Reducing sugar	+	+
Proteins	+	+

“+” indicates mildly present, “++” indicates moderately present, “+++” indicates abundantly present, “-” indicates absent.

Table 2. Antimicrobial activity of different concentrations of ethanol leaf and root extracts of *d. filix-mas*.

		IZD (mm)					
		400 mg/ml	200 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml
Leaf extract	<i>P. aeruginosa</i>	8.5 ± 0.5	4.5 ± 0.5	-	-	-	-
	<i>S. aureus</i>	5 ± 0.0	4.5 ± 0.5	4 ± 0.00	3 ± 0.00		
	<i>E. coli</i>	-	-	-	-	-	-
Root extract	<i>P. aeruginosa</i>	-	-	-	-	-	-
	<i>S. aureus</i>	2 ± 0.00	1.5 ± 1.5	-	-	-	-
	<i>E. coli</i>	-	-	-	-	-	-

Values are expressed as mean ± Standard error of mean (SEM), n =3. IZD: Inhibitory zone diameter. “-”: No inhibition was found.

Table 3. Showing average wound diameter and wound contraction of animals treated with, placebo, povidone iodine and extracts of *d. filix-mas*.

Wound area (cm) and wound contraction (%)								
Group	Baseline (cm)	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21
Control	3.15 ± 0.16	2.46 ± 0.11 (21.92%)	2.62 ± 0.23 (16.77%)	1.44 ± 0.31 (54.13%)	1.13 ± 0.33 (64.17%)	0.77 ± 0.32 (75.41%)	0.55 ± 0.31 (82.4%)	0.42 ± 0.28 (86.59%)
Povidone iodine	2.45 ± 0.21	2.06 ± 0.11 (15.92%)	1.92 ± 0.08 (21.63%)	1.07 ± 0.10 (56.41%)	0.97 ± 0.17 (60.24%)	0.78 ± 0.19 (68.33%)	0.50 ± 0.20 (79.76%)	0.27 ± 0.13 (89.06%)
LE, 1.25%	3.08 ± 0.05	2.68 ± 0.10 (12.81%)	2.58 ± 0.08 (15.99%)	2.16 ± 0.29 (29.71%)	1.52 ± 0.26 (50.46%)	1.19 ± 0.26 (61.38%)	0.79 ± 0.20 (74.19%)	0.42 ± 0.15 (86.35%)
LE, 2.50%	2.71 ± 0.17	2.28 ± 0.14 (15.87%)	2.36 ± 0.22 (13.06%)	1.55 ± 0.18 (42.8%)	1.46 ± 0.14 (46.27%)	1.22 ± 0.18 (54.83%)	0.81 ± 0.23 (69.96%)	0.75 ± 0.16 (72.18%)
LE, 5.0%	3.37 ± 0.17	2.52 ± 0.07 (25.06%)	2.16 ± 0.21 (35.87%)	1.63 ± 0.21 (51.6%)	1.26 ± 0.27 (62.53%)	0.91 ± 0.17 (72.98%)	0.48 ± 0.64 (85.69%)	0.30 ± 0.07 (91.03%)
LE, 10.0%	3.06 ± 0.13	2.18 ± 0.05 (28.98%)	2.01 ± 0.13 (34.40%)	1.28 ± 0.17 (58.16%)	0.70 ± 0.14 (77.15%)	0.44 ± 0.10 (85.70%)	0.19 ± 0.05 (93.86%)	0.11 ± 0.03 (96.54%)
RE, 1.25%	3.62 ± 0.15	2.57 ± 0.05 (28.9%)	2.78 ± 0.23 (23.15%)	1.79 ± 0.15 (50.50%)	1.47 ± 0.21 (59.34%)	0.83 ± 0.18 (77.02%)	0.58 ± 0.29 (83.87%)	0.44 ± 0.32 (87.73%)
RE,	2.92 ± 0.33	2.35 ± 0.31	2.46 ± 0.22	1.80 ± 0.29	1.16 ± 0.42	1.02 ±	0.89 ±	0.41 ± 0.28

2.50%		(19.40%)	(15.63%)	(38.45%)	(60.32%)	0.61 (64.98%)	0.67 (69.57%)	(85.88%)
RE, 5.0%	3.23 ± 0.15	2.50 ± 0.20 (22.5%)	1.98 ± 0.13 (38.75%)	1.30 ± 0.19 (59.83%)	0.75 ± 0.18 (76.75%)	0.41 ± 0.11 (87.29%)	0.26 ± 0.09 (91.82%)	0.18 ± 0.06 (94.48%)
RE, 10.0%	2.87 ± 0.08	2.30 ± 0.10 (19.83%)	1.85 ± 0.21 (35.49%)	1.27 ± 0.13 (55.88%)	0.66 ± 0.15 (76.97%)	0.51 ± 0.15 (82.39%)	0.44 ± 0.17 (84.76%)	0.35 ± 0.17 (87.75%)

Values are expressed as mean ± Standard error of mean (SEM), n =5. LE: leaf extract, RE: Root extract. Italicized values in parenthesis indicate doses and durations where higher activity was observed.

Table 4. Bacterial count of wounds treated with various concentrations of ethanol leaf and root extracts of *d. filix mas*.

	<i>Staph aureus</i> Mean surface count (x 10 ³) / CFU/mL			<i>E.coli</i> Mean surface count (x 10 ³) / CFU/mL		
	Day 10	Day 20	Reduction in bacteria count (%)	Day 10	Day 20	Reduction in bacteria count
Control	62.00 ± 15.72	128.20 ± 70.27	0.00	0.20 ± 0.20	0.00 ± 0.00	100.00
Povidone	50.40 ± 13.27	66.60 ± 58.70	0.00	4.40 ±3.30	60.00 ± 60.00	0.00
L.E, 1.25%	66.00 ± 12.32	38.00 ± 12.13	42.42	137.80 ± 66.80	136.00 ± 67.11	1.31
L.E, 2.5%	103.40 ± 49.74	56.60 ± 8.26	45.26	135.80 ± 67.68	72.60 ± 57.69	46.54
L.E, 5.0%	73.80 ± 10.56	35.00 ± 3.89	52.57	151.20 ± 61.01	45.00 ± 8.02	70.24
L.E, 10.0%	170.00 ± 53.27	19.40 ± 6.48	88.59	211.20 ± 54.38	28.60 ± 9.46	86.46
RE, 1.25%	57.80 ± 11.53	6.20 ± 1.83	89.27	300.00 ± 0.00	17.60 ± 5.97	94.13
R.E, 2.5%	68.20 ± 9.9	14.80 ± 4.27	78.30	208.00 ± 56.66	70.40 ± 57.52	66.15
R.E, 5.0%	156.40 ± 59.04	19.40 ± 5.71	87.60	189.20 ± 67.96	71.80 ± 57.22	62.05
R.E, 10.0%	163.60 ± 56.40	24.40 ±8.76	85.09	248.00 ± 52.00	16.00 ± 4.53	93.55

Values are expressed as mean ± Standard error of mean (SEM), n =5. CFU: Colony forming unit.

Table 5. Effects of ethanol leaf and root extracts of *d. filix-mas* on body weight of animals exposed to excision wounds.

	Initial body weight (g)	Final body weight (g)	Weight gain (%)
Control	128.84 ± 3.97	136.82 ± 7.23	5.83
Povidone	125.58 ± 7.11	139.42 ± 5.58	9.93
L.E, 1.25%	144.14 ± 3.30	158.94 ± 5.61	9.31
L.E, 2.5%	127.30 ± 4.60	156.24 ± 7.46	18.52
L.E, 5.0%	132.98 ± 6.31	159.52 ± 11.52	16.64
L.E, 10.0%	138.50 ± 7.23	175.42 ± 9.31	21.05
RE, 1.25%	120.66 ± 6.95	134.50 ± 11.76	10.29
R.E, 2.5%	146.98 ± 7.44	177.60 ± 10.68	17.24
R.E, 5.0%	175.76 ± 11.20	193.80 ± 9.57	9.31
R.E, 10.0%	158.50 ± 2.96	174.58 ± 5.03	9.21

Values are expressed as mean ± Standard error of mean (SEM), n =5.

ONLINE SUBMISSION

www.ijps.ir