ORIGINAL ARTICLE

In Vitro Antioxidant and Antimicrobial Activity of Polyherbal Formulation

Rohit Kumar[1], Anania Arjuna[2], Diksha[1], Reena Gupta[1], Sanchit Mahajan[3], Saurabh Satija[1], Meenu Mehta[1]

[1]Department of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India, [2]Department of Medical Lab Technology, Lovely Professional University, Phagwara, Punjab, India, [3]Prime Healthcare, San Diego, California, USA

Abstract

Context: Antioxidants play a major role in protecting the body against oxidative stress that is associated with many chronic diseases and disorders including chronic wounds. Plants are the richest source for antioxidant and are effective in the management of oxidative stress, caused by free radical damage. Wound healing and antimicrobial potential are also attributed to the antioxidant potential of drugs. **Aim:** The aim of this study is to carry out the antioxidant and antimicrobial activity of given polyherbal formulation (PHF) to correlate with the wound healing potential of the formulation. **Materials and Methods:** Antioxidant potential of the PHF was evaluated by the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method. Agar well diffusion method was used to determine its antimicrobial activity against the *Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Klebsiella aerogenes.* **Results:** Results of the study demonstrated that PHF exhibited significant antioxidant activity. Antibacterial activity of polyherbal formula was evaluated against the four pathogenic microorganisms in which it showed mild-to-moderate antimicrobial activity against the *E. coli and K. aerogenes,* while mild antimicrobial activity against the *P. aeruginosa and P. vulgaris.* **Conclusion:** Results of this study suggested that PHF can be used for the treatment of wound infections due to its marked antioxidant and antimicrobial activity.

Key words: Free radical, oxidative stress, polyherbal formulation

INTRODUCTION

Skin acts as a protective barrier for the body which protects the body from the external environment. Epidermal layer of skin can be damaged due to the wound[1] which is defined as the disruption of the integrity of the skin by various factors such as pressure, trauma, animal or insect bites, and mechanical abrasions. Invasion of various pathogenic microorganisms at the wounded tissue results in severe chronic wound infection. Anatomical and functional integrity of tissue gets disrupted due to this chronic wound infection. Worldwide, it is estimated that at least 6 million people suffer from chronic wound infections every year.[2]

Wound healing is natural phenomenon by which body restores the cellular and functional continuity of tissue.[3] Appropriate wound healing is necessary to regain the functional and anatomical status of the damaged tissue that got disturbed due to wound. Various complex biochemical events are involved in appropriate healing of wound. These events are divided into three phases: Inflammatory phase, proliferative phase, and remodeling phase.[4] However, various endogenous and exogenous factors such as microbial infection on wound, oxidative stress induced by the reactive oxygen species, poor blood supply to the wound area, nutritional deficiency such as Vitamin C, and zinc result in untimely healing of wound.[5]

Wound infection with the pathogenic microorganisms such as *Staphylococcus aureus, Escherichia coli,* and *P. aeruginosa* results in delay of physiological events involved in the healing of wound.[6] Pathogenic microorganisms invade the open wounds and increase the susceptibility of systemic infections on wounded tissue. Formation of unpleasant exudates and

Address for correspondence:
Dr. Saurabh Satija, Department of Pharmaceutical Sciences, Lovely Professional University, Phagwara - 144 411, Punjab, India.
Phone: +91-7206226042.
E-mail- saurabhsatija87@gmail.com

Received: 09-02-2018
Revised: 27-03-2018
Accepted: 13-04-2018

toxins occurs rapidly in the infected wound that will lead to the destruction of regenerating epidermal cells.[7]

Reactive oxygen species (ROS) such as hydrogen peroxide and superoxide anion can modulate the healing of wounded tissue by stimulating the motility of cells, accelerating the production of inflammatory cytokines, and increasing the formation of new blood vessels. However, the excess production of the free radicals can impair the wound healing by causing damage to DNA, RNA, and proteins. Other than this, excess production of free radicals can also delay the inflammatory phase involved in wound healing by downregulating the level of pro-inflammatory cytokines.[8] Hence, the medicinal plants having marked antioxidant and antimicrobial properties can be used as an effective wound healing agent. Combining the several medicinal plant extract in one formulation often gives more promising pharmacological and therapeutic effect than using a single medicinal plant. A formulation containing several medicinal plants extract is known as polyherbal formulation (PHF). Due to their effective medicinal and therapeutic properties, such formulations are used all over the world over the conventional antibiotics having various side effects. However, still, various herbal therapies, toxicological studies, and *in vitr*o evaluation have not been carried out. There is current need to evaluate the mechanism of action and efficacy of this PHF using scientific methods.[9] Hence, this study aimed to determine the antioxidant and antimicrobial activity of the PHF to prove its efficacy in the treatment of wound infections.

The given PHF is a mixture of several medicinal plant extracts such as *Karanj Beej Oil, Jafi, Neem, Sariva Sativa, Glycyrrhiza glabra, Rubia cordifolia, and Patol Patra*. All medicinal plant extracts present in PHF are a rich source of phytoconstituents such as flavonoids and tannins having strong antioxidant and antimicrobial activity. Various studies demonstrated the wound healing activity of the medicinal plant extract present in the PHF.[10,12,13] The purpose of the present study was to evaluate the antioxidant and antimicrobial effect of the combination of the medicinal plant extract present in given PHF. In this paper, we report the results of the studies to develop the new safer wound healing PHF having significant antioxidant and antimicrobial activity.

MATERIALS AND METHODS

Collection of PHF

PHF was obtained as gift sample from the Shree Dhantwantri herbals.

Chemicals and instruments

2, 2-diphenyl-picrylhydrazyl (DPPH), ascorbic acid, methanol, and dimethyl sulfoxide (DMSO) were obtained from HiMedia. All the chemicals used were of analytical grade. Ultraviolet (UV) visible spectrophotometer used was from Shimadzu.

Biological materials

Four bacterial cultures were used in the study: *Klebsiella aerogenes, P. aeruginosa, E. coli,* and *Proteus vulgaris* which were obtained from the microbial type culture collection and Gene Bank. The microorganisms were maintained on nutrient agar and Mueller-Hinton.

Evaluation of antioxidant activity by the DPPH radical scavenging method

DPPH radical scavenging method is *in vitro* conventional model widely used to access the antioxidant efficacy of compounds. DPPH, a N- centered stable free radical, has strong ability to accept electrons. It exhibits a strong absorbance at 520 nm which disappears when DPPH accepts electrons from the antioxidant compound or reducing agent to become a stable diamagnetic molecule. As a result of this redox reaction, the color of DPPH solution changes from purple to yellow. This change in color and decrease in absorbance of DDPH solution are taken as an indication of the antioxidant potential of tested compounds.[14] It reacts with an reducing agent (AH) based on the following reaction [Figure 1].

Gupta *et al.* used the method with some modifications to estimate the DPPH radical scavenging activity of the sample with some modifications.[15] First, 0.1mM solution of DPPH in methanol was prepared. After this, this solution (3 ml) was added to the 3 ml of PHF solution in DMSO at different concentration (10, 20, 40, 60, 80, and 100 ug/ml). Then after, this mixture was shaken vigorously and allowed to stand for 30 min. After 30 min, absorbance was measured at 517 nm using UV spectrophotometer. Ascorbic acid was used as reference standard, and the same procedure was used to measure its DPPH radical scavenging activity. The half maximal inhibitory concentration (IC_{50}) value of sample PHF and sample was calculated using the log dose inhibition curve. IC_{50} value is defined as the value which is required to inhibit the 50% of the DPPH free radical. The percent DPPH scavenging effect was calculated using the following equation:

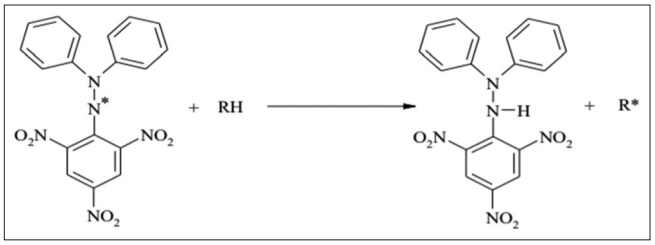

Figure 1: 2, 2-diphenyl-picrylhydrazyl reduction by an antioxidant

$$\frac{\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Sample}}}{\text{Absorbance}_{\text{Control}}} \times 100$$

Where control is DPPH in methanol

Evaluation of antimicrobial activity by agar diffusion method

Antibacterial activity of the PHF was tested against the Gram-negative pathogenic microorganisms *(K. aerogenes, P. aeruginosa, E. coli,* and *P. vulgaris)*. First, Mueller-Hinton Broth agar media was prepared, and then, tested microbial was spread over the entire agar surface. Then with the usage of sterile cork borer, a hole of diameter 6–8 mm was punched aseptically into the well, and then, a volume (1 ml) of PHF was introduced into the well. After this, depending on the test microorganism, agar plates were incubated under the suitable conditions. PHF diffuses into the agar media and inhibits the growth of microbial strain.[11] DMSO was used as positive control in this study. After the incubation, results of antimicrobial activity were expressed as the diameter of zone of inhibition surrounding the walls containing the tested PHF.

RESULTS

Antioxidant activity

The percentage of total antioxidant activity of the PHF is shown in Figure 2. The PHF exhibited the antioxidant activity of 90% at 100 ug/ml, whereas for ascorbic acid (standard) was found to be 75.16 at 100 ug/ml. The IC_{50} value of the PHF and ascorbic acid was found to be 3.118 ug/ml and 23.32 ug/ml, respectively.

Antimicrobial activity

The *in vitro* antimicrobial property of the PHF is presented in the Table 1 and Figures 3-6. The antibacterial activity of the PHF was significantly higher than the control group in which DMSO was introduced into the well of Petri plates. Formulation exhibited mild-to-moderate zone of inhibition against the *E. coli* (2.5 mm) and *K. aerogenes* (2 mm), while mild against the *P. aeruginosa* (1 mm) and *P. vulgaris* (1.5 mm).

Table 1: Zone of Inhibition of PHF against the pathogenic microorganisms

Microbial strain	Zone of inhibition in mm
E. coli	2.5 mm
P. aeruginosa	0.1 mm
P. vulgaris	1.5 mm
K. aerogenes	02 mm

E. coli: Escherichia coli, P. aeruginosa: Pseudomonas aeruginosa, P. vulgaris: Proteus vulgaris, K. aerogenes: Klebsiella aerogenes, PHF: Polyherbal formulation

DISCUSSION

Various scientific studies have reported that herbal plants are a good source of antioxidant and antimicrobial compounds and play a wide role in the treatment of the chronic wound infections globally.[16,17] These plants have been indicated to possess highly antioxidant properties such as reduction of DPPH radical and highly antimicrobial activity against the various pathogenic microorganisms due to the presence of

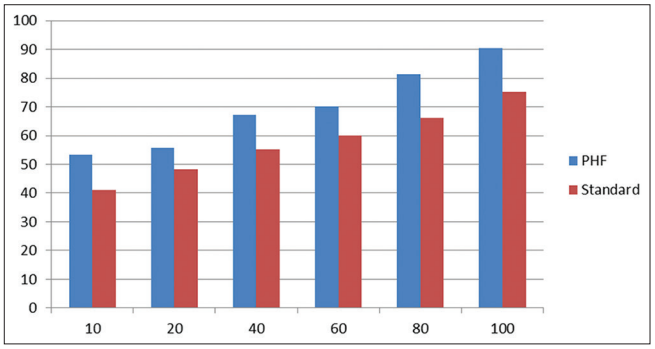

Figure 2: % 2, 2-diphenyl-picrylhydrazyl radical scavenging activity of sample (polyherbal formulation) and standard (ascorbic acid)

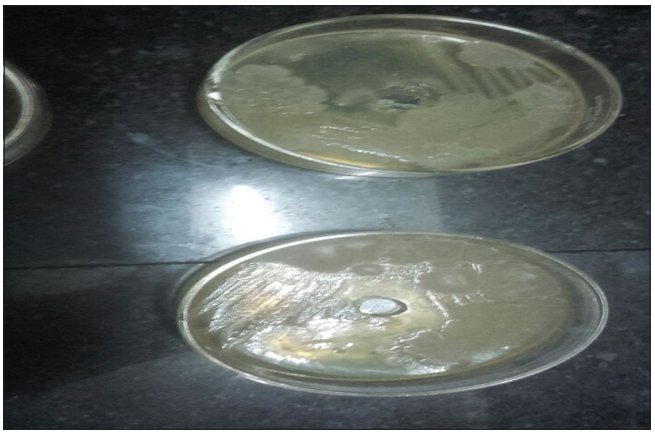

Figure 3: Antimicrobial activity against the *Escherichia coli*

Figure 4: Antimicrobial activity against the *Proteus vulgaris*

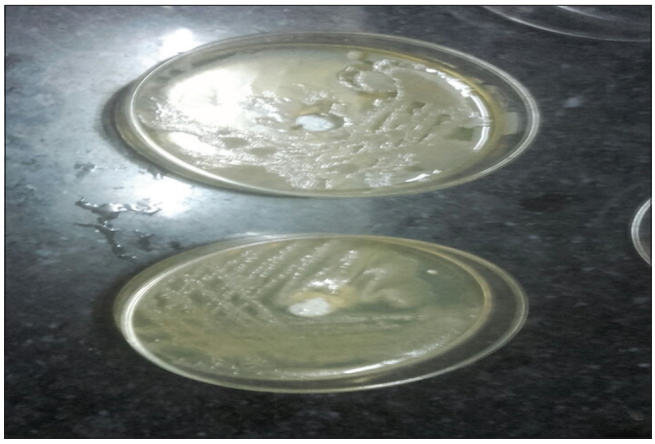

Figure 5: Antibacterial activity against the *Klebsiella aerogenes*

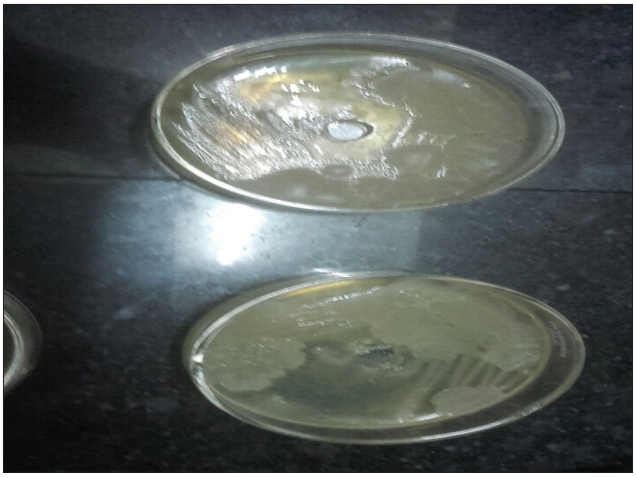

Figure 6: Antibacterial activity against the *Pseudomonas aeruginosa*

phytoconstituents having high free radical scavenging activity and highly antimicrobial properties. Therefore, *in vitro* model DPPH radical scavenging assay and agar diffusion assay were used in this study to evaluate the antioxidant and antimicrobial potential of PHF and to elucidate the wound healing mechanism of formulation.[18]

Excess production of ROS induces oxidative damage by various mechanisms, such as severe destruction to antioxidant enzymes, causing damage to the biological macromolecules such as DNA and RNA, and impairment in the cell signaling pathway ultimately leads to cell death or apoptosis. Various antioxidant compounds used to reduce oxidative stress induced by free radicals acts by several mechanisms such as chelating the free radicals, free radical scavenging mechanisms, modifying the level of antioxidant enzymes and by their reducing potential. DPPH free radical scavenging assay is widely used *in vitro* antioxidant assay used for the evaluation of antioxidant potential of compounds. DPPH is a free radical having the strong ability to accept the electrons to become a stable diamagnetic molecule.[20] Hydrogen-donating ability of the PHF was thought to be responsible for its significant DPPH radical scavenging activity. The decrease in absorbance of DPPH solution on adding PHF is due to the strong reaction that occurs between the formulation having antioxidant potential and DPPH solution. This reaction results in the scavenging of the DPPH radical which can be visualized as change in the color of DPPH solution from purple to yellow on adding PHF. The significant results of the PHF with DDPH *in vitro* antioxidant test provide evidence that it reducing potential is high and it can be effective as scavengers of free radical.[21] Various lines of studies concluded that Gram-negative bacteria are more resistant than the Gram-positive bacteria toward the conventional antibiotics that are used for their treatment.[19] Agar diffusion method is the most widely used technique to assess the effect of antibiotics against the both Gram-positive and Gram-negative bacteria.[22,23] Based on the results of antibacterial activity evaluation by the agar diffusion method, it is concluded that PHF is highly effective against the *E. coli and K. aerogenes*, but mild to moderately effective against the *P. aeruginosa and P. vulgaris*

CONCLUSION

The results of the study suggested that PHF possessed significant antioxidant and antimicrobial activity. Due to its high antioxidant and antimicrobial properties, it can be used as an effective wound healing agent.

REFERENCES

1. Shevchenko RV, James SL, James SE. A review of tissue engineered skin bioconstructs available for the skin reconstruction. J R Soc Interfac 2010;20:229-40.
2. Kumar K, Singh KK, Asthana AK. Ethno therapeutics of bryophyte *Plagiochasma appendiculatum* among the Gaddi Tribes of Kangra Valley, Himachal Pradesh, India. Pharmaceutical Bio 2010;38:353-6.
3. Lazarus GS, Cooper DM, Knighton DR. Definitions and guidelines for assessment of wounds and evaluation of healing. Arch Dermatol 1994;130:489-93.
4. Hess T. Checklist for factors affecting wound healing. Adv Skin Wound Care 2011;24:192-4.
5. Bowler P, Duerden B, Armstrong DG. Wound microbiology and associated approaches to wound management. Clin Microbiol Rev 2011;14:244-69.
6. Mertz P, Ovington L. Wound healing microbiology. Dermatol Clin 2011;24:739-45.
7. Reddy MS, Mondal S, Ghos D, Ramakrishna SG. Evaluation of healing potential of *Achyranthes aspera* (*Amaranthaceae*) seeds in excision, incision, dead space and burn wound model-an *in-vivo* study. Pharmacog J 1999;4:300-10.
8. Rao PS, Kalva S, Yerramilli A, Mamidi S. Free radicals and tissue damage: Role of antioxidants. Free Rad Antiox 2011;1:1-6.

9. Aslam MS, Ahmad MS, Mamat AS, Ahmad MZ, Salam F. an update review on polyherbal formulation: A global perspective. Syst Rev Pharm 2017;7:35-41.
10. Kausik B, Chattopadhyay I, Banerjee RK, Bandyopadhyay U. Biological activities and medicinal properties of Neem (*Azadirachta indica*). Curr Sci 2002;82:1336-45.
11. Magaldi S, Mata-Essayag S, Hartung de Capriles C. Well diffusion for antifungal susceptibility testing. Int J Infect Dis 2004;8:39-45.
12. Shivhare Y, Patel JR, Soni P. Healing efficacy of *Trichosanthes dioica* roxb on dead space wounds. Int J Innov Drug Discov 2014;4:22-4.
13. Patil M, Saini R. Anticonvulsant activity of methanolic extract of *Jasminum grandiflorum* Linn in experimental animals. Res J Pharm Biolog Chem Sci 2014;3:43-9.
14. Lalitha TP, Jayanti P. Study of antioxidant activity of ethanolic extract of fresh *Eichhornia crassipes* (Mart.) Solms. Pharm Sin 2012;3:271-7.
15. Gupta M, Karmakar N, Sasmal S. *In vitro* antioxidant activity of aqueous and alcoholic extracts of polyherbal formulation consisting of *Ficus glomerata* Roxb. and *Symplocos racemosa* Roxb. stem bark assessed in free radical scavenging assays. Int J Pharmacog Phytochem Res 2017;9:181-9.
16. Balouiri M, Sadiki M, Ibnsouda SK. Methods for *in-vitro* evaluating antimicrobial activity: A review. J Pharm Anal 2017;6:71-9.
17. Fouche G, Afolayan AJ, Wintola OA, Khorombi TE, Senabe J. Effect of the aqueous extract of the aerial parts of *Monsonia angustifolia* E. Mey. Ex A. rich., on the sexual behaviour of male wistar rats. BMC Complement Altern Med 2015;15:343-50.
18. Kipkore W, Wanjohi B, Rono H, Kigen G. A study of the medicinal plants used by the marakwet community in Kenya. J Ethnobiol Ethnomed 2014;10:24-46.
19. Silhavy TJ, Kahne D, Walker S. The bacterial cell envelope. Cold Spring Harb Perspect Biol 2011;2:110-8.
20. Narendhirakannan RT. Evaluation of antibacterial, antioxidant and wound healing properties of seven traditional medicinal plants from India in experimental animals. Asian Pac J Trop Biomed 2011;6:1245-S53.
21. Nain P, Kumar A, Sharma S, Nain J. *In vitro* evaluation of antimicrobial and antioxidant activities of methanolic extract of *Jasminum humile* Leaves. Asian Pac J Trop Med 2011;10:804-7.
22. Baba SA, Malik SA. Evaluation of antioxidant and antibacterial activity of methanolic extracts of *Gentiana kurroo* royle. Saudi J Biol Sci 2014;21:493-8.
23. Mehta M, Satija S, Kalsi V. *In vitro* antioxidant evaluation of *Psidium guajava* strem extracts. Int J Drug Dev Res 2011;3:352-5.

Source of Support: Nil. **Conflict of Interest:** None declared.