Copyright © 2014 By IYPF
All rights reserved
Open Access Contents
Int. J. Drug Dev. & Res.
| October - December 2014 | Vol. 6 | Issue 4 | ISSN 0975-9344 |
www.ijddr.in

Full Length Original Research Paper

Evaluation of antimicrobial, antioxidant and wound healing Activity of different fractions of methanolic Extract of *Nerium oleander* Linn

Susanta Kumar Rout*

Durga Madhab Kar

Laxmidhar Maharana

[a] Siksha O Anusandhan deemed to be University, School of Pharmaceutical Sciences, Kalinga Nagar, Ghatikia, Bhubaneswar, Dist: Khurda, Odisha, India. Pin - 751003

Corresponding Authors:
School of Pharmaceutical Sciences, Siksha O Anusandhan University, At: Kalinga Nagar, PO: Ghatikia, Bhubaneswar, Dist: Khurda, Odisha, India. Pin – 752003
E-mail: susanta.rout81@gmail.com
susanta_rout@rediffmail.com

Abstract:

AIM: To investigate the antimicrobial, antioxidant and wound healing activities of different fractions of the methanolic extract of the leaves of *Nerium Oleander* Linn .

METHODS: Antimicrobial activities were carried out by the minimum inhibitory concentration (MIC) through disc diffusion methods and MBC, the wound healing studies were carried out using ether anaesthetized rats in two different wound models Incision and Excision at two different concentrations(5% and 10%w/w). The free radical scavenging activity was studied *in vitro* by measuring DPPH, Hydrogen peroxide scavengering activity, Superoxide free radical (O_2) and Nitric oxide (NO) free radical scavenging activity with reference to standard antioxidant ascorbic acid.

RESULTS: The test compounds like crude methanolic extract (both concentrations) and different fractions like chloroform, methanol and ethyl acetate Significantly increase in tensile strength and the rate of wound contraction compared to the control and nitrofurazone. Antimicrobial activities were evaluated against seven microorganisms namely. *E. faecalis, S. aureus, A. baumannii, E. coli, P.merabilis, P.aeruginosa,* showed significant activity with MIC and MBC respectively. Different fungal strains like *C.albicans and A.niger* are not inhibited by different test compounds. The different extracts and fractions of these plants showed remarkable antioxidant activity.

CONCLUSIONS: The results revealed that the crude methanolic extract, methanol and ethyl acetate fraction produces remarkable wound healing property due to their antimicrobial and antioxidant activities by possessing the active compounds such as flavonoids, terpenes, alkaloids and saponins.

Keywords: *Nerium oleander* Linn, Antimicrobial, antioxidant, wound healing activity

1. Introduction

Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being. Medicinal plants represent a rich source of antimicrobial agents and natural antioxidants [1, 2]. Many of the plant materials used in traditional medicines are readily available in rural areas at relatively cheaper than modern medicines [3]. Approximately 80% of the world inhabitants rely on traditional medicine for their primary health care and play an important role in the health care system of the remaining 20% of the population [4]. The World Health Organization (WHO) is encouraging, promoting and facilitating the effective use of herbal medicine in developing countries for health programs. It has been proved that various plants extracts possess bacteriostatic and bactericidal effects, and most of these plants contain many active compounds. Consequently, they are multipurpose drugs at the same time and have formed the basis of sophisticated traditional medicine system and natural products make excellent leads for new drug development [5-7]. In recent years there has been a growing interest to evaluate plants possessing antimicrobial activities for various diseases [8]. Reactive oxygen species (ROS) are an entire class of highly reactive molecules derived from the metabolism of oxygen. Reactive oxygen species (ROS) including free radicals such as superoxide anion radicals (O_2^-), hydroxyl radicals (OH^-) non-free radicals such as H_2O_2, Singlet Oxygen (O_2) along with various forms of active oxygen are involved in

© 2014 Susanta Kumar Rout et al, publisher and licensee IYPF. This is an Open Access article which permits unrestricted noncommercial use, provided the original work is properly cited.

various physicochemical processes in the body and aging [9]. Free radicals are implicated in a large number of chronic degenerative diseases, inflammation, cataract, atherosclerosis, rheumatism, arthritis, ischemia, etc.[10]. However, they may also cause great damage to cell membranes and DNA, inducing oxidation that causes membrane lipid peroxidation, decreased membrane fluidity, and DNA mutations leading to cancer, degenerative, and other diseases. Antioxidant-based drugs/formulations for the prevention and treatment of complex diseases have appeared during the last 3 decades [11]. This has attracted a great deal of research interest in natural antioxidants. A number of studies have been reported dealing with antimicrobial screening of extracts of medicinal plants. Plant derived drugs have become a popular alternative medicine in developing countries. The potential of higher plants as a source of new drugs is still largely unexplored; hence last decade witnessed an increase in the investigation on plants as sources of new biomolecules for human disease management [12]. Wound healing is a process by which a damaged tissue is restored as closely as possible to its normal state and wound contraction is the process of shrinkage of area of the wound. It mainly depends on the repairing ability of the tissue, type and extent of damage and general state of the health of the tissue. The granulation tissue of the wound is primarily composed of fibroblast, collagen, edema, and small new blood vessels. Wound infection is one of the most common diseases in developing countries because of poor hygienic conditions [13]. Research on wound healing agents is one of the developing areas in modern biomedical sciences and many traditional practitioners across the world particularly in countries like India and China have valuable information of many lesser known hitherto unknown wild plants for treating wounds and burns [14, 15].

N. oleander is an important medicinal plant of family Apocynaceae commonly known as Kaner is large glabrous evergreen shrub with milky juice. This plant grows in Mediterranean region up to Iran and India. Leaves are three, shortly stalked, coriaceous, 10-15 cm long, linear lanceolate with dark green colour. Flowers are salver-shaped pink or white scentless without any fragrance [16]. From the genus Nerium, a number of derivative inaccessible metabolites have been reported. Triterpines, pregnanes, cardenolides, cardiac glycoside were isolated and characterized. [17]. *Nerium oleander* is an evergreen shrub (or small tree) that grows to approximately 6 m. It is widely cultivated as an ornamental shrub or as an informal hedge in warm-temperate and dry subtropical regions. This plant which is known to contain active cardiac glycosides is used in the treatment of cardiac asthma. It may have positive effects in patients with leimyosarcoma and prostate / breast cancer. It is also used as diuretic, anti-inflammatory agent, anti-parasitic and for neurological disorders and cardiac abnormalities. The present study focused on the phytochemical analysis, antimicrobial activity, antioxidant and wound healing activity of methanolic extract and different fractions of crude methanolic extract of *Nerium oleander* Linn.

2. Materials and Methods

Plant Material

Nerium Oleander Linn leaves were collected from Anandapur, Keonjhar district of Odisha, India. The Leaves were authenticated in the Department of Biosciences, Sardar Patel University, Gujarat. The

Covered in Scopus & Embase, Elsevier Int. J. Drug Dev. & Res., October-December 2014, 6 (4): 241-251
© 2014 Susanta Kumar Rout et al, publisher and licensee IYPF. This is an Open Access article which permits unrestricted noncommercial use, provided the original work is properly cited.

plants were collected in bulk and washed with running tap water to remove, adhering soil and dirt particles and then shade dried. A voucher specimen was deposited at the school of pharmaceutical science, SOA University, Bhubaneswar, Odisha. The dried plant materials were coarsely powered and stored in airtight, non-toxic polyethylene bags until used.

Preparation of Extract and Fractions

The powdered leaves of *Nerium Oleander* Linn was extracted with petroleum ether (60 – 80 ºC) for 72h to de-fat it and then the residue plant materials were macerated using methanol as solvent with constant stirring. The solvent incorporating the extractives were filtered and the marc pressed to squeeze out residual extractives. This process was repeated thrice to achieve complete extraction. The extracts obtained during the three cycles were combined and reduced to 1/8th of its original volume in a rotary evaporator at 45 °C and then lyophilized in a freeze dryer to obtain the yield. The extract was again dissolved in distilled water and then successively extracted by the following solvents with increasing polarities; chloroform, ethyl acetate and methanol. The so obtained different fractions were concentrated dried and preserved for further study.

Phytochemical screening give positive tests for alkaloids, glycosides, saponins, Flavonoids, carbohydrates, tannins, phenolic compounds, protein, and fats. All the extracts of plant leaves were prepared 10% w/v in normal saline consisting of 0.1% propylene glycol.

Evaluation of Antimicrobial Activity

Disc Diffusion Method

The antimicrobial activity of the extracts was carried out by disc diffusion test. Antibacterial activity of all the test drugs was carried out by cup-plate method. In this method, cups or discs of standard diameter are made in the nutrient agar medium, containing standard bacterial inoculums. The test compounds were introduced into the discs and the diameter of the zone of inhibition was measured. The test compounds were evaluated for their antibacterial activity against. *S. aureus, B. subtilis, (gram-positive), E. coli, P. aeruginosa (gram negative)* following agar diffusion method of assay [18, 19]. The test organisms were sub-cultured using nutrient agar medium. The tubes containing sterilized medium were inoculated with respective bacterial strain. After incubation at 37±1 ^{0}C for 24 hours, they were stored in refrigerator. Thus, stock cultured was maintained. Bacterial inoculum was prepared by transferring a loopful of stock culture to nutrient broth (100ml) in control flask (250ml). The flasks were incubated at 37±1^{0}C for 18 hours before the experimentation. Solution of the test compounds were prepared by dissolving 10mg each in 10ml of dimethyl formamide (analytical grade) (1000 µg/ml conc.). A reference standard from gram-positive and gram-negative bacteria was made by dissolving accurately weighed quantity of Ampicillin (100 µg/ml) for Gram positive and Gram negative bacteria, griseofulvin (20 µg/ml) for antifungal activity respectively in dimethyl formamide separately. Further, dilution was made with dimethyl formamide itself to obtain a solution of 100 µg/ml. [20].

Minimum Inhibitory Concentration (Mic) Method

The MIC method was applied on extracts that proved their high efficacy against microorganisms by the disk diffusion (Kirby-Bauer) method. The highest dilution of a plant extract that still retains an inhibitory effect against the growth of a microorganism is known as MIC. The complete

© 2014 Susanta Kumar Rout et al, publisher and licensee IYPF. This is an Open Access article which permits unrestricted noncommercial use, provided the original work is properly cited.

protocol of the MIC test is found in the M7-T2 publication of the National Committee for Clinical Laboratory Standards [21]. Briefly, each selected plant extract was subjected to a serial dilution using sterile nutrient broth medium as a diluent. Each plant extract dilution was inoculated with 20 µl of an individual microorganism present in its log phase. All inoculated dilutions were set at 37°C for 24 h. The highest dilution of the plant extract that retained its inhibitory effect resulting in no growth (absence of turbidity) of a microorganism is recorded as the MIC value of the extract. A control experiment was run in parallel to study the impact of the solvent itself (without plant components) on growth of the nine test organisms. Each solvent (water or methanol) was diluted in a similar pattern with sterile nutrient broth, as indicated above, and inoculation by microorganisms followed by incubation were done similarly.

Anti-Oxidant Activity Study of the Extracts

The antioxidant activity of the crude methanolic extract and fractions from *N. Oleander* Linn was determined by *in vitro* models. The *in vitro* methods include Diphenyl-picryl-hydrazyl (DPPH) radical, Superoxide free radical (O_2), Peroxide radical (H_2O_2), and Nitric oxide (NO) free radical scavenging activity with reference to standard antioxidant ascorbic acid.

DPPH Free-Radical Scavenging activity

The free radical scavenging activity of the metabolite, based on the scavenging activity of the stable 1,1- diphenyl-2-picrylhydrazyl (DPPH) free radical was assayed according to the protocol [22]. Briefly, different concentrations of the extracts and ascorbic acid were prepared. Various concentration of test solution in 0.1ml was added to 0.9 ml of 0.1 mM solution of DPPH in methanol. Control was 100µl methanol and 100µl DPPH solution. After 30 minute of incubation at room temperature, the reduction in the number of free radical was measured, reading the absorbance at 517nm. Ascorbic acid was used as reference standard. The scavenging activity of the samples corresponded to the intensity of quenching DPPH. Ascorbic acid was used as reference standard. The compounds with antiradical activity changed color as yellow from the purple-blue.

Quantitative Determination of the DPPH Radical Scavenging Activity

The antioxidant activity of the *N.Oleander* Linn methanolic extract and different fractions were evaluated spectrophotometrically following the DPPH method. Different concentrations of the extracts and fractions 100, 200 and 500 µg/ml) were prepared and mixed 1 ml of them with 2 ml of a freshly prepared DPPH solution (0.01mM); then, each particular sample was mixed thoroughly and kept in the dark for 30 minutes, at room temperature. After that, each mixture was tested for the DPPH radical scavenging activity by reading the absorbance at 517 nm on a UV-VIS spectrophotometer. As blank was used a solution prepared by mixing 1 ml of methanol with 2 ml of the DPPH solution (0.01mM) and reading at the same wavelength. In addition, to eliminate the absorbance of the crude extracts at this wavelength, blank samples were prepared with 1 ml of each extract and 2 ml of methanol. The antioxidant activity percentage was calculated following the formula:

Antioxidant activity (%) = ((AC -AE) /AC) x 100

Where AC is the absorbance of a DPPH solution without extract, AE is the absorbance of the tested extract and fractions, which is equal to the absorbance of the plant extract plus the DPPH (0.01mM) minus the blank extract absorbance

© 2014 Susanta Kumar Rout et al, publisher and licensee IYPF. This is an Open Access article which permits unrestricted noncommercial use, provided the original work is properly cited.

(28). As standard ascorbic acid at different concentration 5-25 µg.ml-1 was used. The EC50 value, defined as the concentration of the sample leading to 50% reduction of the initial DPPH concentration, was calculated from the linear regression of plots of concentration of the test extracts against the mean percentage of the antioxidant activity [23].

Superoxide (O_2) Free-Radical Scavenging activity

Measurement of superoxide anion (O_2) scavenging activity of extracts and fractions was based on the method with slight modification [24]. $O2^-$ radicals are generated non-enzymatically in Phenazine methosulphate–Nicotinamide adenine dinucleotide phosphate (PMS–NADH) systems by the oxidation of NADH and assayed by the reduction of NBT. In this experiment, the superoxide radicals were generated in 1 mL of Tris-HCl buffer (16 mM, pH 8.0) containing nitro blue tetrazolium (NBT) (50 µM) solution and NADH (78 µM) solution. The reaction was started by adding PMS solution (10 µM) to the mixture. The reaction mixture was incubated at 25°C for 5 min, and the absorbance at 560 nm in a spectrophotometer was measured against blank samples. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The percentage inhibition of superoxide anion generation was calculated using the following formula:

$(\%)\ I = (A_0 - A_1) / (A_0) \times 100$

Where A0 was the absorbance of the control and A1 was the absorbance of extract and the standard compound.

Peroxide free radical (H_2O_2) scavenging activity

Scavenging of H_2O_2 by the extract and fractions was determined. One millilitre of *C. siamea* flower extract solution (prepared in phosphate buffered saline (PBS)) was incubated with 0.6 ml of 4mM H_2O_2 solution (prepared in PBS) for 10 min. The absorbance of the solution was measured at 230 nm against a blank solution containing the extract without H_2O_2. The concentration of H_2O_2 was spectrophotometrically determined from absorption at 230 nm using the molar absorptivity [25].

Nitric Oxide Free Radical (No) Scavenging Activity

Nitric oxide (NO) was generated from sodium nitropruside (SNP) and measured by Greiss reaction [26]. SNP in aqueous solution at physiological pH instinctively generates NO, which intermingles with oxygen to generate nitrite ions that can be anticipated by Greiss reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride). Scavengers of NO with oxygen leading to reduced production of NO. SNP (5 mM) in phosphate buffer saline (PBS) was mixed with different concentration of (100-500 µg/mL) drug dissolved in suitable solvent and incubated at 25°C for 150 min. The above samples were reacted with Greiss reagent. The absorbance of chromophore created during diazotization of nitrite with sulphanilamide and following coupling with naphthyl ethylene diamine was read at 546 nm and referred to the absorbance of standard solution of potassium nitrite treated in the same way with Greiss reagent [27].

Excision Wound Model

An impression was made on the dorsal thoracic region 1cm away from vertebral column and 5cm away from ear using a round seal of 2.5cm diameter on the anaesthetized rat. The skin of impressed area was excised to the full thickness to obtain a wound area of about 300 mm2 diameters. Hemostasis was achieved by blotting the wound with cotton swab soaked in normal saline. Contractions, which contribute for wound

© 2014 Susanta Kumar Rout et al, publisher and licensee IYPF. This is an Open Access article which permits unrestricted noncommercial use, provided the original work is properly cited.

closure in the first two weeks, were studied by tracing the wound on a transparency paper initially. Then an impression was taken on a millimeter scale graph paper, scar area after complete epithelization and time for complete epithelization in days was evaluated to calculate the degree of wound healing. The parameters were studied wound closure, epithelization time and scar features. The observation of the percentage wound closure were recorded on 4th , 8th, 12th, and 16th post wounding day and also for epithelization and size and shape of scar area [28] .

Incision Wound Model

In the incision model, the rats were anesthetized by anaesthetic ether and two longitudinal paravertebral incisions of 6cm length were made through the skin and cutaneous muscle at a distance of about 1.5cm from the midline on each side of the depilated back. After the incision, the parted skin was sutured 1cm apart using a surgical thread (No. 000) and curved needle (No. 11). The wounds were left undressed. The extracts were given by oral route once a day, till complete healing. The sutures were removed on eighth post-wound day. The skin-breaking strength of the 10-day-old wounds was measured by the method of Lee [29, 30].

Statistical Data Analysis

Results were expressed as mean ± SEM. All the results were analyzed by One-way Analysis of Variance (ANOVA) followed by Dunnett's test. The level of significance was set at $P<0.05$.

3. Result

The phytochemical screening (Table. 1) of the different fractions and crude methanolic extact of *N.Oleander* Linn revealed the presence of glycosides, steroids, flavonoids, terenoids, saponins and reducing sugar. The crude methanolic extracts and fractions of the plant was studied against both gram-negative, gram-positive bacteria and fungus related to their zone of inhibition, MIC and MBC. The results of the antimicrobial screening of the methanol extracts and different fractions of *N.Oleander* Linn was shown in (Tables 2 and 3). The results were recorded as presence or absence of zones of inhibition around the well as well as MIC and MBC. The inhibitory zone around the well indicated the absence of bacterial growth and it was reported as positive and absence of zone as negative. The crude methanolic extracts of leaves of *N.Oleander* Linn Showed moderate to high antimicrobial activity against all the tested microorganisms except A.niger and C.Albicans. Crude methanolic extract and different fractions were used to assess the in-vitro antioxidant activity. The antioxidant activity of Plant extract and fractions were determined by different *in vitro* methods such as, the DPPH free radical scavenging assay and reducing power methods in (Tables 4). All the assays were carried out in triplicate and average values were considered. Antioxidant scavengering activity was studied using 1, 1—diphenyl, 2-picrylhydrazyl free radical. Various concentration of test solution in 0.1ml was added to 0.9 ml of 0.1 mM solution of DPPH in methanol. Methanol only (0.1ml) was used as experimental control. After 30 minutes of incubation at room temperature, the reduction in the number of free radical was measured, Ascorbic acid was used as reference standard. The crude methanolic extract along with methanolic, ethayl acetate and chloroform fractions showed a concentration dependent antiradical activity by scavengering DPPH radical.

© 2014 Susanta Kumar Rout et al, publisher and licensee IYPF. This is an Open Access article which permits unrestricted noncommercial use, provided the original work is properly cited.

Crude methanolic extract was found to be more potent compared to other fractions. The observations made in the present study showed that the extract of *N.Oleander* Linn leaves exhibited good scavengering of H_2O_2 in the biological system, thus preventing the stress induced by progressive increase in malondialdehyde and other free radicals which cause oxidative damages. All various fractions and crude methanolic extract of these plants were capable of reducing DNA damage comparing to control. The percentage inhibition of nitric oxide generation by different fractions and extracts of *N.Oleander* Linn leaves at different concentration were compared with standard. All test compounds exhibited potential inhibiting activity against NO generation. Nitric oxide is a potent pleiotropic mediatorof physiological processes. The antioxidant activity of crude methanolic extract of *N.Oleander* Linn showed highest inhibition of nitric oxide generation which is compared with standard ascorbic acid. The effect of crude methanol extract and different fractions ointment on excision and incision wound model was continued up to 16 days. The results of excision and incision wound study are shown in (Table 5 and 6). From the results, it was observed that the wounds treated with test formulation show increase in tensile strength compared to untreated control group thus promoting wound healing. A significant increase in tensile strength of the indicative of improved collagenation which significantly contributes to better and effective healing.

4. Discussion

The different parts of the plant such as root, bark and leaves of *N.Oleander* Linn has been used for thousands of years for its medicinal properties [31]. It is rich in a wide variety of secondary metabolites such as glycosides, phytosterols, proteins, saponins and phytosterols. In this connection the present study on the methanolic extract and the different fractions of crude methanolic extract was conducted to evaluate the antimicrobial activity of leaves. Phytomedicines can be used for the treatment of diseases as is done in case of Unani and ayurvedic system of medicines, a natural blue print for the development of new drugs [32]. The MIC values of those extract and fractions which gave positive results during prelim. screening were determined by Tube Dilution Method. The antimicrobial study was conducted using different micro organisms such as Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, A. Baumannii, Candida albicans. aspergilus Niger. The extract and fractions of *N.Oleander* Linn showed the higher zone of inhibition. Though the growth of Staphylococccus aureus, Pseudomonas aeruginosa, Staphylococcus epidermidis, Pseudomonas aeruginosa are controlled by *Nerium oleander*, it indicates that they could inhibit the activity of bacteria which causes diarrhoea, polymixin and typhoid respectively. Polyphenols, including flavonoids, forms a large group of naturally occurring components of the plant kingdom and are present in every part of the plants. These compounds are of considerable interest in various fields such as food, pharmacy and medicine because of wide range of biological activities including antioxidant activity. The antioxidant efficacy of phenolic compounds is chiefly due to their redox potential. These compounds are known to act as reducing agents (free radical terminators), hydrogen donors, metal

© 2014 Susanta Kumar Rout et al, publisher and licensee IYPF. This is an Open Access article which permits unrestricted noncommercial use, provided the original work is properly cited.

chelators and singlet oxygen quenchers. Since it has been shown that phenolic compounds of plant kingdom are one of the most effective antioxidative constituents. Flavonoids are polyphenolic compounds and consist of flavones, flavonols, flavanols, flavanone and flavanonols. These compounds represent the majority of plant secondary metabolites and have shown to possess remarkable health promontory effects such as anti-inflammatory, antioxidant, antimicrobial, anticancer and others [33]. Interception of free radicals or other reactive species is mainly by radical scavenging and is caused by various antioxidants like vitamins C and E, glutathione, other thiol compounds, carotenoids, flavonoids, etc. While at the repair and reconstitution level, mainly repair enzymes are involved [34, 35]. In general, peroxyl radicals cause chain reactions in lipids, proteins and DNA. The high reactivity of the representative peroxyl radical shows that the possible mechanism behind the observed protection of these biomolecules by DA may be through scavenging of secondary radicals. The soluble free radical DPPH is well known as a good hydrogen abstractor yielding DPPH-H as by product. Thus, the scavenging of DPPH radicals by phenols are most of the time very effective. All the plant extracts used in this study were primarily screened against the test microorganisms by the Different Methods like Disc diffusion and MIC methods. The relative efficacy of some commonly used antibiotics were compared with plant extract discs. Wound healing is a process by which a damaged tissue is restored as closely as possible to its normal state and wound contraction is the process of shrinkage of area of the wound. Collagen is a major protein of the extracellular matrix and is the component that ultimately contributes to wound strength. Tannins promote the wound healing through several cellular mechanism; chelation of the free radicals and reactive species of oxygen, promoting contraction of the wound and increasing the formation of capillary vessels and fibroblasts and including keratinocyte proliferation , but do not act on the differentiation towards cornified cells [36, 37]. The collagen composed of amino acid (hydroxyproline) is the major component of extra cellular tissue, which gives strength and support. Breakdown of collagen liberates free hydroxyproline and its peptides. On the basis of the results finding in the present investigation, it is concluded that the crude methanolic extract of *N.Oleander* Linn produces highest wound healing activity plant extract and Fractions. The animals treated with crude methanolic extract of plants showed highest wound healing potency treatment was continued upto 16th days. The present studied also showed ethyl acetate fraction and chloroform fraction possesses a good wound healing activity, Further investigations are needed for identification of active principles responsible for the wound healing activity. The present investigation offers a scientific support to the traditional healer account in use of the plants *Nerium Oleander* Linn. The present study suggests that the antimicrobial, antioxidant and wound healing activity can be enhanced by the use of crude methanolic extracts of *N.Oleander* Linn. The experimental results indicate that the methanolic extract of *N.Oleander* Linn produced significant result in woundhealing activity through tropical application.

5. Conclusion

Covered in Scopus & Embase, Elsevier Int. J. Drug Dev. & Res., October-December 2014, 6 (4): 241-251
© 2014 Susanta Kumar Rout et al, publisher and licensee IYPF. This is an Open Access article which permits unrestricted noncommercial use, provided the original work is properly cited.

Drugs from plants have a long history in both traditional and modern societies as herbal remedies

or crude drugs and as purified compounds. The present study revealed that the selected Plant extract and some fractions of the crude extracts produced antimicrobial, antioxidant and wound healing efficacy with dose dependent manner. The observed activities of leave extract might be attributed to the presence of secondary metabolites such as flavonoids and phenolic compounds. The leaves can be used to prevent oxidative damage caused by free radicals and to treat infections caused by pathogenic bacteria not to fungus. Further studies with purified constituents are needed to understand the complete mechanism of wound healing activity of the test plants. However, it needs further evaluation in clinical settings before consideration for the treatment of different disorders.

6. Acknowledgments

The research project was supported by University Grant Commission. The authors wish to thank

Prof. Dr. Sudam Sci, Dean, SPS and SOA University for providing facilities to conduct work and moral support.

7. References

1) B. Mahesh and S. Satish, "Antimicrobial activity of some important medicinal plant against plant and human pathogens," *World J. Agri.Sci.* 2008; 4: 839-843,.

2) B. Halliwell, R. Aeschbach, J. Lo Liger and O.I. Aruoma, "The characterization of antioxidant," *Food Chem. Toxicol.* 1995; 33: 601-617,.

3) Mann A, Banso A, Clifford LC, *et al.* An Antifungal property of crude plant extracts from *Anogeissus leiocarpus* and *Terminalia avicennioides* (J) *Tanzania J Health Res*, 2008; 10: 34-38,.

4) G.M. Cragg, M.R. Boyd, R. Khanna, R. Kneller, T.D. Mays, K.D. Mazan, D.J. Newman, E.A. Sausville. International Collaboration in drug discovery and development, the NCT experience. Pure Appl. Chem. 1999;71: 1619-1633.

5) H.S. Lee, Growth inhibitory effect of various medicinal plants against lactic acid and harmful intestinal bacteria. Food Sci.Biotech. 2000; 9: 52-56.

6) P.S. Negi, G.K. Jayaprakasha, M. Jagan, K.K. Sakariah, Antibacterial activity of tumeric oil: by product from Curcumin manufacture. J. Agric. Food Chem. 2000; 47: 297- 300.

7) D.J. Newman, G.M. Cragg, K.M. Snader, The influence of natural products upon drug discovery. Nat. Prod. Res. 2007; 17: 215-234.

8) Clark AM and Hufford CD, Discovery and development of novel prototype antibiotics immunodeficiency syndrome, *In*: Human Medical Agents from Plants, by A Douglas Kinghorn and ManuelF Balandrin (Eds), American Chemical Society (ACS Symposium Series 534), Washington DC, 1993; 228-241.

9) Finkel, T., Holbrook, N.J.,. Oxidant, oxidative stress and the biology of ageing. Nature 2000; 408; 239–247.

10) Dro¨ ge, W. Free radicals in the physiological control of cell function. Physiol. Rev. 2002; 82: 47–95.

11) Devasagayam TPA, Tilak JC, Boloor KK et al. Review: Free radicals and antioxidants in human health: Curr Stat Fut Prosp. JAPI. 2004; 52: 794-804.

12) D.S. Grierson, A.J. Afolayan, Antibacterial activity of some indigenous plants used for the treatment of wounds in the Eastern cape, South Afr. J.Ethnopharmacol. 1999; 66: 103-106.

13) Perumal Samy R and Ignacimuthu S, Antibacterial activity of some folklore medicinal plants used by tribals in Western Ghats of India, J Ethanopharmacol, 2000; 69: 63-71.

© 2014 Susanta Kumar Rout et al, publisher and licensee IYPF. This is an Open Access article which permits unrestricted noncommercial use, provided the original work is properly cited.

14) Senthil Kumar M, Sripriya R, Vijaya Raghavan H and Sehgal P. Wound Healing Potential of *Cassia fistula* on Infected Albino Rat Model. *J. Surg. Res.* 2006; 131: 283-289.

15) Kumara Swamy HM. Wound healing activity of embelin isolated from the ethanol extract of leaves of *Embelia ribes* Burm. *J. Ethnopharmacol.*, 2007;109: 529- 534.

16) Portillo A, Vila R, Freixa B, Adzet T and Canigueral S, Antifungal activity of Paraguayan plants used in traditional medicine, J Ethanopharmacol, 2001;76: 93-98.

17) Tart, Charles T, Major Psychedelic Drugs: Altered states of consciousness, 3rd ed., San Francisco (Harper), 1990; 454-460.

18) Bauer, A. W., Kirby, W. M. M., Sherries, J. C. & Turck, M.. Antibiotic susceptibility testing by single disk method. *American Journal of Clinical Pathology*, 1966; 45: 493-496.

19) National Committee of Clinical Laboratory Standard. Document M2-M3 method for dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, 1993; 3rd ed. Approved Standards. Villanova, PA, USA.

20) Misra, S. B. & Dixit, S. N. Antifungal properties of leaf extract of *Ranunculus sceleratus. L. Experientia*, 1978; *34:* 1442-1443.

21) Blois, MS, "Antioxidant determinations by the use of a stable free radical," *Nature,* 1958; 181: 1199-150.

22) W. B. Williams, M. Cuvelier, C. Berset, "Use of a free radical method to evaluate antioxidant activity," *Lebensm-Wiss Technol.*1995; 28: 25- 30,.

23) A. B. Ribeiro, V. S. Bolzani, M. Yoshida, L. S. Santos, M. N. Eberlin, D. H. S. Silva, "A new neolignan and antioxidant phenols from *Nectandra grandiflora," J. Braz. Chem. Soc*. 2005;16: 526-530,.

24) Oktay, M., Gulcin, I., Kufrevioglu, O.I., Determination of in vitro antioxidant activity of fennel (Foeniculum vulgare) seed extracts. Lebensmittel Wissenchaft und Technol. 2003;36: 263-271.

25) Ruch, R.J., Cheng, S.J., Klaunig, J.E., Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis 1989;10: 1003–1008.

26) Green, L.C., Wagner, D.A., Glogowski, J., Analysis of nitrate, nitrite and 15 (N) nitrate in biological fluids. Anal Biochem 1982;126: 131-138.

27) Werner S, Breededen M, Hubner G, Greenhalgh D G and Longaker M T. "Introduction of keratinocyte growth factor expression is reduced and delayed during wound healing in the genetically diabetic mouse", J Investig Dermatol., 1994; 103:469.

28) Ehrlich HP, Hunt TK. "The effect of cortisone and anabolic steroids on the tensile strength of healing wounds", Ann Surg., 1968;167:324.

29) Chua LS, Latiff NA, Lee SY, Lee CT, Sarmidi MR, Aziz RA, *et al*. Flavonoids and phenolic acids from Labisia pumila (Kacip Fatimah) (J). *Food Chemistry*, 2011; 127: 1186-1192

30) Lee KH. "Studies on the mechanism of action of salicylate retardation of wound healing by aspirin", J Pharma Sci., 1968; 57:1042-3.

31) Hill AF, Economic botany. A textbook of useful plants & plant products. 1952, 2nd edn. McGarw-Hill Book Company Inc, New York.

32) Dhar, M. L., M.M.Dhar, B.N.Dhawan and C.Roy,. Indian J. Exp. Biol. 1968; 6: 232.

33) Chua LS, Latiff NA, Lee SY, Lee CT, Sarmidi MR, Aziz RA. Flavonoids and phenolic acids from Labisia pumila (Kacip Fatimah). Food Chemistry 2011; 127: 1186-1192.

34) H. Sies, In: Antioxidants in disease, Mechanisms and Therapy, Academic Press, New York, 1996.

35) B. Halliwell, O.I. Aruoma (Eds.), DNA and Free Radicals, Boca Raton Press, 1993.

36) Fernandez,O.,Capdevila, J.Z., Dalla, G. and Melchor, G., Fitoterapia., 2002;73: 564.

37) Deters, A., Dauer, A., Schnetz, E., Fartasch, M. and Hensel, A. Phytochemistry., 2001; 58: 949.

© 2014 Susanta Kumar Rout et al, publisher and licensee IYPF. This is an Open Access article which permits unrestricted noncommercial use, provided the original work is properly cited.

Article History: ----------

Date of Submission: 16-12-2014

Date of Acceptance: 29-12-2014

Conflict of Interest: NIL

Source of Support: NONE

SJR SCImago Journal & Country Rank

Powered by SCOPUS™

© 2014 Susanta Kumar Rout et al, publisher and licensee IYPF. This is an Open Access article which permits unrestricted noncommercial use, provided the original work is properly cited.