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WOUND HEALING ACTIVITY OF ETHANOLIC POLYHERBAL EXTRACT IN WISTER RATS

S.Vetriselvan*[1], Rusliza Basir[2], U.Subasini[1], C.Velmurugan[3]

[1*]Department of Pharmacology, Master skill University College of Health Sciences, Malaysia.
[2]Pharmacology Unit, Faculty of Medicine and Health Sciences, University Putra Malaysia, Malaysia.
[3]Department of Pharmacology, Sri Krishna Chaithanya College of Pharmacy, Gangannagaripalle, Madanapalle, Andhra Pradesh, India.

ABSTRACT

There are many plants and their products that have been mentioned or used in the Indian traditional system of medicine and have shown wound healing activity. Some herbal extracts has been confirmed in human and animal models of wound healing. The plants credited with wound healing action had major chemical constituents like glycosides, alkaloids, flavonoids, triterpenes, mucilages, polysaccharides, oils, vitamins, saponins, glycoproteins, amino acids, peptides and proteins. The polyherbal extract contains the plant parts are whole plant of *Adiantium capillus*, seeds of *Astercantha longifolia*, fruits of *Callicarpa macrophylla*, bark of *Ficus benghalensis*, aerial parts of *Melia azedarach*. In this present study ethanolic polyherbal extract is screened for its phytochemical evaluated in wound healing activity in Wister rats by excision wound model using povidone iodine as a reference standard. From the results it was found that Polyherbal ethanolic extract possess significant wound healing action when compared to control and equipotent wound healing activity when compared to standard povidine iodine.

KEYWORDS

Herbal plant, *Adiantium capillus*, *Astercantha longifolia*, *Callicarpa macrophylla*, *Ficus benghalensis*, *Melia azedarach* and wound healing activity.

Author for Correspondence:
S. Vetriselvan,
Department of Pharmacology,
Masterskill University College of Health Sciences, Malaysia.

Email: vetricology@gmail.com

INTRODUCTION

Wound is perhaps an in escapable event in the life of an organism. Healing process begins during the early phase of inflammation, but reaches completion usually after the injurious influence has been neutralized. It should be emphasized that without inflammation wounds would never heal. In

spite of the strenuous efforts by various scientists all over the world, very few wound healing drugs have been discovered as an ideal treatment for this disease. So, there is an urge of new wound healing agent.

India having a rich heritage of traditional medicine constituting with its different components like Ayurveda, Siddha and Unani. Botanicals constitute of major part of these traditional medicines. The development of these traditional systems of medicines with the perspectives of safety, efficacy, and quality will help not only to preserve the traditional heritage but also to rationalize the use of natural products in the health care. The main advantages of herbal medicines are their time tested inherent efficacy, less side effects, and low cost and herbal system of medicine is the oldest medical system in existence. In India herbal medicines are widely used to treat diabetic condition[1]. Several marketed herbal products are available in the market for the treatment of wound healing. The amalgam of coordinated events that constitute the process of wound healing is quite complex. The steps in the procession of wound healing include inflammation, the fibroblastic phase, scar maturation, and wound contracture[2, 3]. Wound contracture is a process that occurs throughout the healing process, commencing in the fibroblastic stage[3].

The inflammatory phase occurs immediately following the injury and lasts approximately 6 days. The fibroblastic phase occurs at the termination of the inflammatory phase and can last up to 4 weeks. Scar maturation begins at the fourth week and can last for years[4]. An analogous system depicts the 4 phases as hemostasis, inflammation, granulation, and remodeling in a continuous symbiotic process[4]. The polyherbal extract contains the plant parts were whole plant of *Adiantium capillus*, seeds of *Astercantha longifolia*, fruits of *Callicarpa macrophylla*, bark of *Ficus benghalensis*, aerial parts of *Melia azedarach*. The present study is an attempt to investigate the effect of alcoholic of polyherbal extract on wound healing animal model in Wister rats.

MATERIAL AND METHODS

Coarsely powdered materials of the plants *Adiantum capillus* (Whole plant), *Astercantha longifolia* (seeds), *Callicarpa macrophylla* (fruits), *Ficus benghalensis* (bark), *Melia azedarach* (aerial parts) were collected from SKM Siddha and Ayurveda Company (India) Limited, Erode.

ASTERACANTHA LONGIFOLIA

Botanical Name(s)	*Asteracantha Longifolia*
Family Name	*Acanthaceae*
Kingdom	*Plantae*
Division	*Magnoliophyta*
Class	*Equisetopsida*
Order	*Lamiales Bromhead*
Family	*Acanthaceae*
Genus	*Asteracantha*
Species	*Asteracantha Longifolia*

Chemical constituents: A. longifolia contains Apignin-7-0-glucoside, 7-0- glucoside, histidine, lysine, phenylalanine, linoleic acid, palmitic acid, stearic acid, xylos, uronic acid, polysaccharides, xylan, protease, lupeol, betulin, phytosterol, ascorbic acid, nicotinic acid etc.

Uses: Roots are sweet, sour, bitter, anti-inflammatory, refrigerant, diuretic, analgesic, haemopoictic, hepatoprotective and tonic. It is useful in inflammations, hyperdipsia, strangury, jaundice and vesical calculi. It is also used in flatulence and dysentery. Leaves are haemopoictic, hepatoprotective, anti-inflammatory, antioxidant, analgesic, antidiabetic, stomachic, ophthalmic, diuretic and liver tonic. It is used in hepatic obstruction, jaundice, arthritis, rheumatism and diseases of urinogenital tract.

CALLICARPA MACROPHYLLA

Botanical Name(s)	*Callicarpa Macrophylla*
Family Name	*Verbenaceae*
Kingdom	*Plantae*
Order	*Lamiales*
Family	*Verbenaceae*
Genus	*Callicarpa*
Species	*Callicarpa Macrophylla*.

Chemical constituents: Four chemicals have been isolated that appear to be the active ingredients; borneol, callicarpenal, intermedeol, and spathulenol. The isolation of abieta-8, 11, 13, 15-tetraen-18-oic

acid, calliphyllin, calliterpenone, 6α-hydroxynidorellol, and isopimaric acid, and the authors observed that several of these same compounds have been reported from members of the Lamiaceae, supporting an alliance with the latter plant family. Xu and coworkers reported the isolation of four new clerodane, diterpenes, pentandralactone and pentandranoic acids A–C.

Uses: The flowers and fruits are bitter, sweet, astringent, acrid, anodyne, stomatitis, expectorant, depurative, anthelmintic, deodorant, digestive, styptic, febrifuge and good tonic. Flowers and fruits are useful in rheumatoid arthritis, asthma, catarrh, anorexia, headache, foul ulcers, flatulence, colic diarrhea, dysentery, skin diseases, burning sensation, excessive sweating, diabetes, vomiting, fever and general debility.

FICUS BENGHALENSIS

Botanical Name(s)	*Ficus Benghalensis*
Family Name	*Moraceae*
Kingdom	*Plantae*
Division	*Magnoliophyta*
Class	*Magnoliophyta*
Order	*Urticales*
Family	*Moraceae*
Genus	*Ficus*
Species	*Ficus Benghalensis*

Chemical constituents: [5] Leaves yield quercetin-3-galactoside, rutin, friedelin, taraxosterol, lupeol, β-amyrin along with psoralen, bergapten and β-sisterol. The bark of Ficus bengalensis presence of 5,7 Dimethyl ether of leucopelargonidin-3-0-α-L rhamnoside and 5,3 dimethyl ether of leucocynidin 3-0-α-D galactosyl cellobioside, glucoside,20-tetratriaconthene-2-one,6-heptatriacontene-10-one, pentatriacontan-5-one, beta sitosterol-alpha-D-glucose, and meso-inositol Earlier, glucoside, 20 tetratriaconthene-2-one, 6-heptatriacontene-10-one, leucopelargonidin have been isolated from the bark of the Ficus bengalensis.

Uses: According to Ayurveda, it is astringent to bowels and very useful in treatment of biliousness, vaginal complains, fever, ulcers, erysipelas, vomiting, inflammations and leprosy. According to Unani system of medicine, latex is maturant, lessens inflammations, aphrodisiac, tonic, vulernary and is useful in piles, nose-diseases, gonorrhea etc. The aerial root is styptic and is useful in syphilis, biliousness, dysentery and inflammation of liver. It acts as an astringent, antidiarrheal, antidysenteric, hemostatic and antihemorrhoidal. The tree is widely planted for shade and its leafs are used for fodder.

MELIA AZEDARACH

Botanical Name(s)	*Melia Azedarach*
Family Name	*Meliaceae*
Kingdom	*Plantae*
Division	*Magnoliophyta*
Class	*Magnoliophyta*
Order	*Sapindales*
Family	*Meliaceae*
Genus	*Melia*
Species	*Melia Azedarach*

Chemical constituents

The bark and root bark contain triterpenoids: toosendanin, isotoosendanin, kulinone, kulactone, kulolactone, methylkulonate, melianodiol, meliantriol, trichilin H, anthraquinone glycosides: 1,8-dihydroxy-2-methylanthraquinone-3-O-β-D-galactopyranoside,1,5-dihydroxy-8 metho-2-methylanthraquinone-3-O-rhamno pyranoside; flavonoids:4',5-dihydroxyflavone-7-O-α-L-rhammo pyranosyl-(1→4)-β-D-glucopyranoside, melianxan thone ;phenolic compounds: hexacosyl ferulate, etc.

Uses: Leaves: leprosy, scrofula, anthelmintic, antilithic, diuretic, deobstruent, resolvent. Root: resolvent, deobstruent. Seeds: rheumatism. Leaves: Leaf extract has insecticidal property (azadirachtin) that repels insects in clothing. The leaves can also serve as feed for goats. Seed oil: The oil is the most active medicinal product of the plant. It is used as antiseptic for sores and ulcers that show no tendency to heal. The powder was subjected to various studies for which the materials and methods presented below.

Extraction Procedure

Equal amount of the weighed coarse powders were mixed and blended. The coarse power was used for the extraction by successive solvent extraction by Soxhlet apparatus using various solvents. The

assembly of soxhlet apparatus as shown in the Figure No.5.

Alcoholic extract

Marc obtained from the above extract was dried and extracted with 2.5litre of ethanol. Then it was filtered and stored in dessicator.

Animals

Healthy, adult Wistar rats of both sexes (150-220g) were obtained from the animal house facility A.M Reddy Memorial College of pharmacy, Narasaraopet, Guntur, Andhra Pradesh. The animals were kept in a well ventilated room and the animals were exposed to 12 hrs day and night cycle with a temperature between 20±3ºC. The animals were housed in large spacious, hygienic polypropylene cages during the course of the experimental period. The animals were fed with water and rat feed ad libitum, supplied by this institution. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals and approved by Animal Ethical Committee of Department Pharmacology, A.M Reddy Memorial College of pharmacy, Narasaraopet, Guntur, Andhra pradesh. (Approval no: 1012/c/06/CPCSEA).

Chemicals and reagent

Beeswax, hard paraffin, cetosteryl alcohol, soft paraffin and other chemicals used in the studies were analytical laboratory grades procured from the following manufactures, Darwin laboratories, Vijayawada, Andhra Pradesh.

Phytochemical Screening

The plant may be considered as a bio-synthetic laboratory, not only for the chemical compounds such as carbohydrates, proteins and lipids that are utilized as food by man but also for a multitude of compounds like glycosides, alkaloids, volatile oils, tannins etc. that exert a physiologic and therapeutic effect. The compounds that are responsible for medicinal property of the drug are usually secondary metabolites. A systematic study of a crude drug embraces, thorough consideration of primary and secondary metabolites derived as a result of plant metabolism[6]. The pharmacological effects of some of the phytochemical constituents are: Glycosides the organic compounds derived from plants are genetically used as cardio-tonic agents, diuretics and sedatives. Tannins are secondary metabolites which are used in the treatment of diarrhea, hyperglycemia and hypertension etc. Alkaloids are a chemically heterogeneous group of natural substances generally used as anti cholinergic, local anesthetics, anti malarial agents.

Qualitative phytochemical screening of plant extracts

The crude extracts of the plant were subjected to chemical tests for the identification of various active constituents like alkaloids, carbohydrates, proteins amino acids, steroids, cardiac glycosides, phenolic and tannins tests were performed as described in reference [7].

Pharmacological studies

Acute toxicity studies[8]

Healthy adult female Wister rats starved overnight were divided into four groups (n=3) and were given different doses of the extract. Group I 2000mg/kg orally, Group II 300mg/kg orally, Group III 50mg/kg orally, Group IV 5mg/kg orally. The animals were observed continuously every five minute, every ten minutes for one hour and at the end of twenty four hours. Since no animal died even in the maximum dose of 2g/kg of body weight, $1/10^{th}$ of it i.e. 200mg/kg of body weight was selected for the study.

Procedure

Preparation of Extract

The polyherbal extract was prepared by adding 1 liter of ethanol thrice, at an interval of two days. The ethanol containing extract, so obtained each time was mixed and later dried at room temperature. The yield of ethanolic polyherbal extract was 4.5 to 5 % (w/w) and 1.6 to 1.8 % (w/w) respectively.

Wound healing activity

Excision wound model[9-12]

Wound healing activity of ethanolic polyherbal extract was assessed using excision wound model. The albino rats of either sex (150-200 gm) were divided in to six groups of five animals each. Test (ethanolic polyherbal extract) were formulated as 5 % w/w ointment for local application, using simple ointment as vehicle.

The various groups were treated as follows:

Group-I: Control (5 % w/w simple ointment applied locally)

Group-II: Standard (5 % w/w povidine iodine ointments applied locally)

Group-III: Polyherbal extract (5% w/w ointment applied locally)

Animals were under light ether anesthesia throughout the surgical procedures. An impression of 2.5 cm diameter (500 sq mm) was made after leaving at least 5 mm space from the ears. The skin of the impressed area was excised carefully to the complete thickness and a wound of 500 sq mm was formed. Homeostasis was achieved by application of normal saline solution. The animals were kept individually in separate cages. The physical attributes of wound healing viz., wound closure (contraction) and epithelization period were recorded. The wound contraction was studied by tracing the raw wound area on a transparent sheet on 4^{th}, 8^{th}, 12^{th} and 16^{th} day. The criterion for complete epithelization was fixed as formation of the scar with absence of raw wound area. The wound area was measured planimetrically by the help of sq. mm scale graph paper. The percentage wound closure was calculated using the following formula:

Wound closure = $[1 – A_d/A_o] \times 100$

Where,

A_o = Wound area on day zero (500 sq. mm)

A_d = Wound area on corresponding days.

The results are tabulated in Table No.2. The results obtained were subjected to statistical analysis using ANOVA followed by Turkey-Krammer Multiple Comparison Test.

RESULTS AND DISCUSSION

Preliminary phytochemical screening

The Polyherbal extract was subjected to chemical tests as per the standard methods for the identification of the various constituents.

Acute toxicity studies

Acute toxicity studies on female rats showed no mortality at a dose of 2000mg/kg, during a time period of 14 days. During the study, no noticeable responses were seen in the rats. This help to predict that it does not contain any type of toxicity and is safe. So 100 mg/kg b.w (1/20th) and 200 mg/kg b.w (1/10th) were selected of that dose for further study.

DISCUSSION

Wounds are the physical injuries that results in an opening or break of the skin. Proper healing of wounds is essential for the restoration of disrupted anatomical continuity and disrupted functional response of the several cell types to injury. Cutaneous wound repair is accompanied by an ordered and definable sequence of biological events starting with wound closure and progressing to the repair and remodeling of damaged tissue[13]. The measurements on 4^{th} day showed that the percentage closure of original excision wound area in solvent control group was 20.6 ± 2.28 sq.mm in Povidine iodine treated group was 46.5 ± 2.11sq.mm and Polyherbal extract treated group was 20.72 ±2.16 sq.mm. The Polyherbal extract and Povidine iodine have significantly promoted the wound healing when compared with control group.

The measurements on 8^{th} day showed that the percentage closure of original excision wound area in solvent control group was 42.7 ± 1.40 sq.mm in Povidine iodine treated group was 69.3 ± 1.06sq.mm and Polyherbal extract treated group was 47.7±1.90 sq.mm. The Polyherbal extract and Povidine iodine have significantly promoted the wound healing when compared with control group.

The measurements on 12^{th} day showed that the percentage closure of original excision wound area in solvent control group was 53.5 ± 3.78 sq.mm in Povidine iodine treated group was 79.6 ± 2.92 sq.mm and Polyherbal extract treated group was 61.3±1.38 sq.mm. The Polyherbal extract and Povidine iodine have significantly promoted the wound healing when compared with control group.

The measurements on 16^{th} day showed that the percentage closure of original excision wound area in solvent control group was 71.7± 2.96 sq.mm in Povidine iodine treated group was 93.3 ± 3.74sq.mm and Polyherbal extract treated group was 84.7±2.58 sq.mm. The Polyherbal extract and

Povidine iodine have significantly promoted the wound healing when compared with control group.

Table No.1: Qualitative phytochemical screening of polyherbal extract

S.No	Plant constituent	Ethanol extract
1	Alkaloids	+
2	Carbohydrates	+
3	Glycosides	+
4	Saponins	-
5	Protein and amino acids	+
6	Phytosterol	+
7	Phenols and tannins	+
8	Flavonoids	+
9	Fixed oils and fat	+

"+" Present, "-"Absent.

Table No.2: Effect of topical application of polyherbal 5 % ointment of ethanolic polyherbal extract on excision (open) wound parameters

S.No	Groups	% Contraction of wound on different days (sq.mm)				Epithelization time in days
		4^{th} day	8^{th} day	12^{th} day	16^{th} day	
1	Control	20.6± 2.28	42.7±1.40	53.5±3.78	71.7±2.96	24± 1.42
2	Povidine iodine	46.5± 2.11***	69.3± 1.06***	79.6± 2.92***	93.3± 3.74***	18.2 ± 0.69***
3	Polyherbal alcoholic extract	20.72± 2.16*	47.7± 1.90*	61.3± 1.384**	84.7± 2.58.**	19.5 ± 1.28**

Values are Mean ± S.E.M., n=5, Where *P<0.05, **P<0.01 and ***P<0.001 vs Control

Figure No.1: Photograph of *Asteracantha Longifolia*

Figure No.2: Photograph of *Callicarpa Macrophylla*

Figure No.3: Photographs of *Ficus Benghalensis*

Figure No.5: Extraction using soxhlet apparatus

CONCLUSION

The present study indicates that polyherbal contains the plant parts are whole plant of *Adiantium capillus*, seeds of *Astercantha longifolia*, fruits of *Callicarpa macrophylla*, bark of *Ficus benghalensis*, aerial parts of *Melia azedarach* are extracted with ethanol. Ethanolic polyherbal extract were subjected for phytochemical screening, from the results Ethanolic polyherbal extract posses various constituents like alkaloids, glycosides, carbohydrates, phenols, phytosterol, proteins and amino acids, fixed oils and tannins. Finally the

Figure No.4: Photograph of *Melia Azedarach*

ethanolic polyherbal extract were screened for wound healing activity by excision wound model using povidine iodine as a reference standard. From the above results it can be concluded that polyherbal extract showed potent wound healing activity when compared to control and showed equipotent activity to that of reference standard povidine iodine on experimentally induced wounds in rats. The recovery of wound is due to the presence of above mentioned chemical constituents might have favored wound healing activity against experimentally induced wounds in rats. Further clinical studies are needed to establish its safety and usefulness in wound patients.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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