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PHYTOCHEMICAL SCREENING AND WOUND HEALING ACTIVITIES OF METHANOLIC EXTRACT OF *BASELLA ALBA* LEAF AND FRUIT FORMULATED IN A SIMPLE OINTMENT BASE

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Abstract

Medicinal plants constitute an important natural wealth of a country. They play a significant role in providing primary health care services to rural people. They serve as therapeutic agents as well as important raw materials for the manufacture of traditional and modern medicine. A large number of plant extracts or decoctions are equally used folklore traditions in India for treatment of cuts, wounds and burns. The methanolic extract of the leaf and fruit of *Basella alba L*, was investigated to determine its total yield, phytochemical constituents and wound healing in groups of Wistar rats by excision model. Bioactive constituents like alkaloids, glycoside, Terpenes, saponins, tannins and flavonoids occur in abundance in the crude aqueous extract. Two test doses of ointment applied to study wound healing i.e 5% and 10%. The parameters studied included rate of % wound contraction and days of complete wound healing. The result of the study related to wound healing was very interesting. There was a progressive decrease in wound area with time, indicating an efficacy of the formulations in healing the induced wounds. The % mean wound contraction area in day 12^{th} was 45.2± 1.4 (control), 47.06± 4.2 (5% Leaf) and 61.62± 3.46 (10% Leaf), 65.24±2.86(5% Fruit), 78.24±3.5 (10% Fruit) and 78.02±2.4 (Povidone iodine ointment). A better healing pattern with complete wound closure was observed in rats treated with 10% methanolic fraction within 12 days while it took 21 days in control rats. There was a significant increase (p□ 0.5, p□0.05, p□ 0.01) in wound contraction from day 4 onwards in all the treated rats except those treated with 10% methanolic fraction in day 4. The present study

thus provides a scientific rationale for the traditional topical application of the ointment of leaf and fruits of *Basella alba L*., on wounds.

Key words: *Basella alba,* Phytochemicals, Wound healing activity, Excision model, methanolic extract.

Introduction

Plants have been used as a source of medicine and which will be used for the treatment. During last two decades revolutionary efforts have been observed in phytotherapeutic treatment of various ailments. It is fascinating to see that when the area of 'Tele medicine' is coming up and probably arrived at the most modern doors, the people living in far-flug villages are still healed at nature's own dispensary with the fast progress of technology, new horizons of medicine are opening with modern scientific and medical technology can give as cost effective and potential new drugs. As such, traditional medicine is of contemporary relevance in India to achieve self-reliance in primary health care. Medicinal plants are the local heritage with global importance (Singh, 2009). Use of plants as a source of medicine has been inherited and is an important component of health care system in India. India is the largest producer of medicinal herb and called 'Medicinal garden of the world' (Suresh et al.,2005). Developed nations besides probingfor traditional medications for the treatment of some serious illness such as heart diseases, high blood pressure, asthma and other problems. The main advantage of using plants as medicines are less toxic, softer than manufactured drugs, cheaper and easy availability Agarwal B and Avinash sani 2008).

The wound may be defined as a loss or breaking of cellular and anatomic or functional stability of living tissues. Healing of wound is a naturalpractice that is initiated by trauma and frequently terminated by scar formation. The wound healing will be progressed in different phases such as coagulation, epithelization, granulation, collegenation and tissue remodeling. In India, there has been interest in the potential of medicinal plant for development of drugs with wound healing properties as taught in a popular form of Indian medicine known as Ayurveda (Jain et al., 2006). Research on drugs that increase wound healing is a developing area in modern

biomedical sciences. Several drugs obtained from plant sources are known to increase the healing of different types of wounds (Biswas and Mukherjee 2003). Medicinal plants are coming into prominence to conventional medicines such as antibiotics which has resulted in the development of resistance in many infectious organisms. Thus, herbal preparations can be more effective than conventional medicines and their non-toxic nature means that they can be administered over long periods (Vinothapooshan and Sundar2010).

Materials And Method

Collection of plant materials:

Nature plants of *Basella alba* was used for this study and it was collected from Komarapalayam, Namakkal. Tamil Nadu in 2015. Authentication was carried out at the Department of Botanical Surveyof India, Southern Regional Centre and Tamil Nadu Agricultural University Campus in Coimbatore, where voucher specimens were deposited. The voucher specimen numbers were assigned as follows: *Basella alba L. (Basella rubra L.) BASELLACEAE*. The plant parts were washed thoroughly with distilled water.

Phytochemical analysis and Soxhlet extraction:

The study has been designed for the evolution of phytochemical screening of Methanolic extract of *Basella alba*.The Leaf, stem and Fruit were Extracted using soxhlet apparatus.

Methods of Extraction:

Normally a solid material containing some of the desired compound is placed inside a thimble made from thick filter paper which is loaded into the main chamber of the soxhlet extractor. The soxhlet extractor is placed onto a flask containing the extraction solvent. The soxhlet is then equipped with a condenser.The solvent is heated to reflux. The solvent vapour travels up a distillation arm and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapour cools and drip back down into the solid material.

The chamber containing the solid material slowly fills with solvent. Some of the desired compound will then dissolve in the warm solvent. When soxhlet chamber is almost full the chamber is automatically emptied by a siphon side arm with the solvent running back down to

the distillation flask. This cycle may be allowed to repeat many times over hours or days.During each cycle a portion of the non-volatile compound dissolves in the solvent. After many cycle the desired compound is concentrated in the distillation flask. The advantage of this system is that instead of many portions of warm solvent being passed through the sample just one batch of solvent is recycled.

After extraction the solvent is removed typically by means of a rotary evaporator yielding the extracted compound. The non-soluble portion of the extract solid remains in the thimble and is usually discarded.The dried plant materials were ground into fine powder in an electric blender and subsequently sieved for obtaining fine powder. 50g of sieved powder was weighed accurately and subjected to extraction in a soxhlet apparatus at room temperature for 48 hours using Methanol successively. Before extraction with the next solvent the power was air dried to remove the adhering solvent. The extract obtained was filtered and concentrated plant extract used for phytochemical screening.

Detection of carbohydrates:

a) Aqueous extracts were treated with 1ml of barford's regent. The solutions were heated in a beaker of boiling water bath gives a red precipitate indicates the presence of reducing sugar.

b) Crude extract was mixed with 2ml of Molisch's reagent and the mixture was shaken property. After that, 2ml of concentrated H_2SO_4 was poured carefully along the side of the test tube. Appearance of a violet ring at the interphase indicated the presence of carbohydrate.

Detection of Glycosides:

a) To the hydrolyzate 1ml of pyridine and few drops of sodium nitroprusside solution were added and then it was made alkaline with sodium hydroxide. The pink colour changes into red shows the presence of glycosides.

b) Hydrolyzate was treated with chloroform and the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. The pink colour changes into red shows the presence of glycosides.

Detection of Proteins and Amino acids:

a) Crude extract when mixed with 2ml of Millon's reagent, white precipitate appeared which turned red upon gentle heating that confirmed the presence of protein.

b) Crude extract when boiled with 2ml of 0.2% solution of Ninhydrin, violet colour appeared suggesting the presence of amino acids and proteins.

c) To the extract equal volume of 5% sodium hydroxide solution and 1% copper solution were added. A violet color formation indicates the presents of amino acids.

Detection of Alkaloids:

a) Filtrates were treated with Dragendroff's reagent. Formation of red precipitate indicates the presence of alkaloids.

b) Filtrateswere treated with Wagner's reagent (Iodine in potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloid.

c) Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids conformed by the formation of yellow coloured precipitate.

Detection of Flavanoids:

a) Crude extract was mixed with 2ml of 2% solution of NaOH. An intense yellow colour was formed which turned colorless on addition of few drops of diluted acid which indicated the presence of flavonoids.

b) Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavanoids.

Detection of Phenols-Tannins:

a) To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

b) Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

c) The ethanol extract of plant material was treated with 5ml of 1% hydrochloric acid. Formation of red precipitate indicates the presence of Phlonatannins.

Detection of Saponins:

a) In a test tube containing about 5 ml of extracts, a drop of sodium bi carbonate solution was added. The mixture was shaken vigorously and kept for 3 minutes. A honey comb like froth was formed and it showed the presence of saponins.

b) Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Detection of Steroids, Vitamins, Terpenoids:

a) Crude extract was mixed with 2ml of chloroform and concentrated H_2SO_4 was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids. Another test was performed by mixing crude extract with 2ml of chloroform. Then 2ml of each of concentrated H_2SO_4 and acetic acid were poured into the mixture. The development of a greenish coloration indicated the presence of steroids.

b) Crude extract was mixed with 2ml of chloroform and evaporated to dryness. To this, 2ml of concentrated H2SO4 was added and heated for about 2 minutes. A grayish colour indicated the presence of terpenoids.

Detection of Quinone, Oxalate:

a) A small amount of extract was treated with concentrated HCL and observed for the formation of yellow precipitate (or colouration).

b) To 3ml portion of extracts were added a few drops of ethanoic acid glacial. A greenish black colouration indicates presence of oxalates.

WOUND HEALING ACTIVITY:

Ethical clearance:

Excision and incision wound models were used to evaluate the wound-healing activity of *Basella alba*. The study was approved by the Institutional Animal Ethical Committee of Nandha College of Pharmacy and Research Institude, Lalitha (V), registered under CPCSEA, India. The

study has been designed for the evalution of wound healing activity of methanolic extract of *Basella alba*.

Chemicals

Povidone iodine cream (PI) (1%w/w), methanol, sterilized cotton were used.

Preparation of 5% and 10% ointment of *Basella alba* (w/w)

Five gram and Ten gram of the methanolic extract of *Basella alba*was mixed 95g of Vaseline to prepare 5% and 10% ointment (w/w). Himax (Indian herbs Research & Supply Co. Ltd. DarraShivpuri, Saharanpur) was used as standard drug.

Animals

Healthy wistar albino rats of either sex and of approximately the same age, weighing about 150-250 g were used for the study. They were fed with standard diet and water ad libitum. They were housed in polypropylene cages maintained under standard conditions (12/12 hr light/dark cycle; 25ºC ± 30ºC, 35- 60% RH).

Selection of dose

For the assessment of cutaneous wound healing activity, dose level was chosen in such a way that, dose was approximately one tenth of the maximum dose during acute toxicity studies (5% and 10%).

Grouping of animals

Animals were divided in to six groups, each group consisting of 6 rats.

Group I:

Received no treatment and served as control

Group II:

Received application of standard drug ointment i.e. Povidin Iodine cream (PI) (1 %w/w).

Group III:

Received application of *Basella alba* leaf extract Wound healing activity (5%).

Group IV:

Received application of *Basella alba* leaf extract Wound healing activity (10%)

Group V:

Received application of *Basella alba* fruit extract Wound healing activity (5%)

Group VI:

Received application of *Basella alba*friut extract Wound healing activity (10%)

Extraction procedure:

The plant material was washed with water and shade dried at room temperature. The dried plant materials were ground into fine powder in an electric blender and subsequently sieved for obtaining fine powder. 75gms of sieved powder was weighed accurately and subjected to extraction in a soxhlet apparatus at room temperature for 48hrs using methanol successively. Before extraction with the next solvent the powder was air dried to remove the filtered and concentrated in rotary flash evaporator. The concentrated plant extract used for wound healing assays.

Experimental Animals:

Male albino rats (150-180g) used in the present study were procured from the small animal breeding station, Mannuthy, Kerala, India. They were housed in polypropylene cages (38×28×10cm) with not more than six animals per cage and maintained under standard environmental conditions (14h dark/10h light cycles; temperature 25±2°c;35-60% humidity, air ventilation) and were fed with standard pellet diet (m/s. Hindustan lever Ltd, Mumbai, India) and

fresh water ad libitum. The animals were acclimated to the environment for two weeks prior to experiment use. Animals were fasted overnight before the experimental schedule, but have free access for water ad libitum. The experiment was carried out according to the guidelines prescribed by animal Welfare Broad and with the prior approval of animal ethic committee.

Preparation of ointments:

To 10g of petroleum jelly 0.5g of sample extract was added and stirred to produce the 5% low dose ointment. The 10% high dose ointment was prepared by stirring 10g of petroleum jelly with 1.0g of sample extract. This 5% and 10% ointment was used for topical application Mohammed .A, Sidik.K and Salmah.I (2011).

Excision wound model:

Wister albino rats (150-180g) were divided into four groups of six animals each (n=6) group I serve as induced and Group II as standard which was topically applied with providence iodine ointment for 21 days after the wound excision. Group III and IV were topically applied with 5% and 10% (W/W) ointments prepared using the sample extract and petroleum jelly for 21 days. The animals were depilated on the back and cutaneous wound of 1.0sq cm were inflicted on the pre-shared sterile dorsal surface of each animal of group I-Group IV under ether anesthesia Sainuddin and Haneefa(2010). Application of drugs was done once a day after cleaning with surgical cotton wool. To assess the area of the area of the healing wound, the length and breadth of the wounds were measured for 21 days using a vernier caliper on days 0, 6, 12, 18 and 21 the wounds were photographed.

Results And Discussion

Plants produce wide array of bioactive molecules or phytochemicals which probably evolved as chemical defense against predation or infection but, are now found to be useful for treatment of various ailments. Due to the myriad of potential benefits they possess, plants have been widely exploited in traditional medicine and their curative potentials are well documented Krishnaiah D., Davit, BonoA and Sarbatly, R.(2009). Although there are no apparent morphological characteristics in the medicinal plants growing with them, yet they possess some special qualities or virtues that make them medicinally important. It has now been established

that the plants which naturally synthesis and accumulate some secondary metabolites like alkaloids, glycosides, tannins, volatile oil, possess medicinal properties.

Phytochemical examinations were carried out formethanolic extract of *B.alba* plant as per methods mentioned by AOAC(1990) and Makker H.P.S, Becker K andSchmook B (1998). Phytochemical screening of different extracts of *Basella alba*. The result indicates the presence of tannin, diterpenes, steroid, saponins, anthroquinone, alkaloid, carbohydrate, glycoside, flavanoid, phenol, aminoacid. The phytochemical analysis results of all extracts were shown in Table I.

Table I: Phytochemical Screening of methanolic Extract of *Basella alba*whole plant

Plant parts / Constituent	Leaves	Fruit	Stem
Carbohydrates	+	+	+
Glycosides	+	+	+
Amino acid	+	+	+
Alkaloid	+	+	+
Flavanoid	+	+	+
Saponins	+	-	-
Steroid	+	+	-
Vitamin	+	+	+
Terpenoids	+	+	+
Phenol	+	-	+
Tannin	+	+	+
Anthroquinone	-	-	+
Oxalate	+	+	+

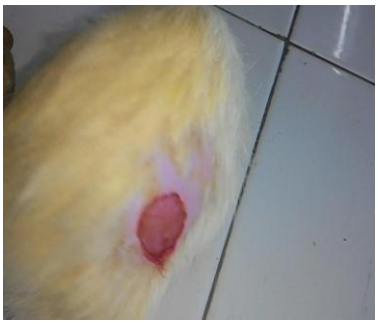
Wound healing activity:

Wounds are referred to as disruption of normal anatomic structure and function. Skin wounds could happen through several causes like physical injuries resulting in opening and breaking of the skin Gerald S.L, Diane M.C, David R.K, David J.M, Roger E.P, George R(1994). The most common symptoms of wounds are bleeding, loss of feeling or function below the wound site, heat and redness around the wound, painful or throbbing sensation, swelling of tissue in the area and pus like drainage RashedAN, Afifi FU, Disi AM,(2003).

The measures of the progress of wound healing induced by the Povidone ointment (1g), Plant extract and methanol (5%) and (10%) and the control rats are shown Plate III, Table III and corresponding to this value a graph is also plotted which is given in the figure. It is observed that the wound contracting ability of the extract ointments were significantly greater than that of the control, and was comparable to that of the reference standard Povidone ointment. The extract ointment produced complete healing on 21st day.

In the excision wound, the *Basella alba*extract significantly increased the collagen, Protein content when compared to the control, standard drug, where as shown in figure I and Table II respectively. When compare to fruit extract shows high healing activity compare to leaf extract of *Basella alba*.

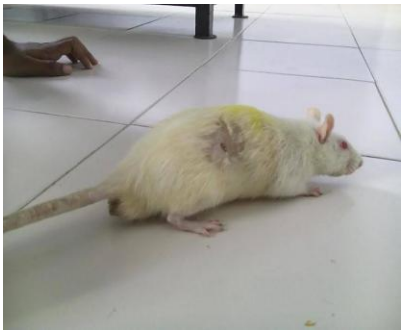
Wound healing activity of methanolic Leaves extract of *Basella alba*



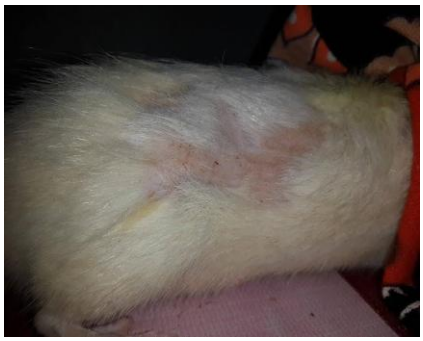
0th day

4th day

8thday

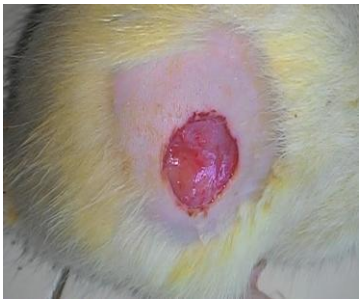


12th day

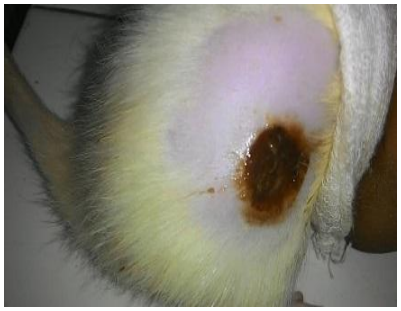


21st day

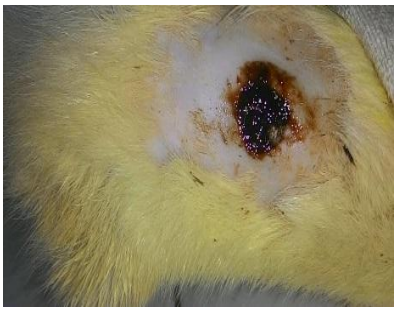
Wound healing activity of methanolic fruit extract of *Basella alba*



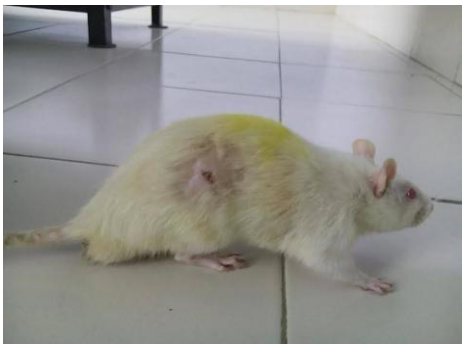
0th day



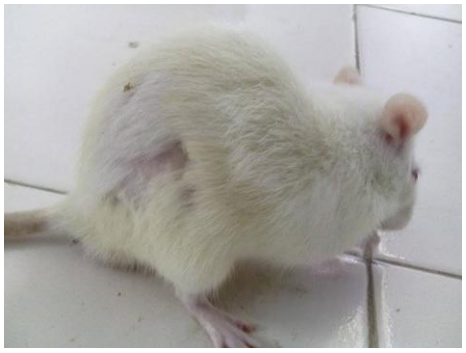
6th day



8th day



12th day



21st day

Table II: Wound healing activity of extract in rats by Excision model

GROUP	Wound contraction (%)				Epithelialization period (day)
	0 day	4 day	8 day	12 day	
GroupI Simple ointment (control)	8.72±0.32	19.6±0.4	36.6±1.2	45.2±1.4	22.4±1.6
Group II Povidine iodine	15.42±1.2**	34.3±2.2**	59.6±4.32**	78.02±2.4**	16.68±0.94**
Group III Leaf (5%)	9.4 ± 1.6^{ns}	18.6±2.4*	38.02 ± 1.8^{ns}	47.06 ± 4.2^{ns}	20.2 ± 0.92^{ns}
Group IV Leaf (10%)	11.3±1.08**	26.82±1.2**	42.66±3.2**	61.62±3.46**	18.62±0.82**
Group V Fruit (5%)	13.5±0.86**	30.24±2.2**	44.24±0.68**	65.24±2.86**	17.92±0.58**
Group VI Fruit (10%)	14.2±0.62**	35.24±0.9**	60.14±1.5**	78.24±3.5**	17.04±0.92**

Values were mean ± SEM, n=6,

$^{ns}P> 0.5$, *$P<0.05$, **$P<0.01$ Vs control(one way ANOVA followed by Dunnett's test).

Conclusion:

To promote the proper uses of herbal medicine and to determine their potential as sources of new drugs, it is essential to study medicinal plants. The curative properties of medicinal plants are mainly due to the presence of various complex chemical substances of different composition. In the present study, we have found that most of the biologically active phytochemicals in the methanolic extract of *B.alba*. Phytochemicals is readily available over the counter from *B.alba*.

There is currently a large and over expanding global population base that prefers the use of natural products in treating and preventing medicinal problems because herbal plants have a rich source of medicinal properties. The plants studied here can be seen as a potential source of useful drugs. Result of this study suggest that the utility of *B.alba* for various nutritional products. Different phytochemicals have been found to possess a wide range of activities which may help in protection against ocular diseases.

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