QUALITATIVE PHYTOCHEMICAL ANALYSIS & INVESTIGATION OF ANTHELMINTIC AND WOUND HEALING POTENTIALS OF VARIOUS EXTRACTS OF *CHROMOLAENA ODORATA LINN*. COLLECTED FROM THE LOCALITY OF MOHUDA VILLAGE, BERHAMPUR (SOUTH ORISSA)

Debashisha Panda*[1], Santosh Kumar Dash[1], Gouri Kumar Dash[2]
College of Pharmaceutical Sciences, At. Mohuda , Berhampur -760002, Orissa[1]
Matushree V.B. Manvar College of Pharmacy, Tumiyani, Rajkot-360440, Gujarat[2]
*Email: Debashisha_panda@rediffmail.com

ABSTRACT

Recently, there has been growing interest in the traditional cures of livestock diseases because of expensiveness of Pharmaceutical products. The self-help approaches in form of traditional medicines, especially from medicinal plants, offer a way out by making use of resources available within the communities themselves. Helminth infections are among the most common infections in man, affecting a large proportion of the world's population. In developing countries they pose a large threat to public health and contribute to the prevalence of malnutrition, anaemia, eosinophilia, and pneumonia. Wounds are generally produced by physical, chemical, thermal, microbial or immunological insult to the tissues. The process of wound healing consists of integrated cellular and biochemical events leading to reestablishment of structural and functional integrity with regain of strength of injured tissues. The fresh leaves and extracts of *Chromolaena odorata* are used as traditional herbal treatments for burns, soft tissue wounds and skin infections. The present study reveals that the methanolic extract of the leaf part of *Chromolaena odorata* Linn has got promising effectiveness in the treatment of helmintic infections as well as wound healing process.

Keywords: *Chromolaena odorata*, anthelmintic, wound-healing, Pheretima posthuma , Swiss Albino rats.

INTRODUCTION

The use of traditional medicines holds a great promise as a easily available source as effective medicinal agents to cure a wide range of ailments among the people particularly in tropical developing countries like India. In this context, the people consume several plants or plant derived formulations to cure helmintic infections (1) and treatment of wounds (2)

Chromolaena odorata, previously called *Eupatorium odoratum* is known to have originated from South and Central America and is commonly called siam weed, triffid weed, bitter bush or jack in the bush (3). It is an herbaceous perennial that grows to a height of three meters in open situation and up to eight meters when assumed a scrambling habitat in the interior forests (4). The fresh leaves and extract of *C. odorata* are used in traditional herbal treatment in developing countries for burns, soft tissue wounds and skin infections (5).

Helminth infections are among the most common infections in man, affecting a large proportion of the world's population. In developing countries they pose a large threat to public health and contribute to the prevalence of malnutrition, anaemia, eosinophilia, and pneumonia. Although the majority of infections due to worms are generally limited to tropical regions, they can occur to travellers who have visited those areas and some of them can develop in temperate climates (6). The gastro-intestinal helminthes becomes resistant to currently available anthelmintic drugs therefore there is a foremost problem in treatment of helminthes diseases (7); hence there is an increasing demand towards natural anthelmintics. Therefore, the present study of various solvent extracts of the leaves of Chromolaena odorata was carried out for anthelmintic activity.

Wound is a breach in the normal tissue continuum, resulting in a variety of cellular and molecular sequelae. Wound may be produced by physical, chemical, thermal, microbial or immunological insult to the tissue. The process of wound healing consists of integrated cellular and biochemical events leading to reestablishment of structural and functional integrity with regain of strength of injured tissue. Clinically, one often encounters non-healing, under-healing or over healing. Therefore the aim of treating a wound is to either shorten the time required for healing or to minimize the undesired consequences. A number of drugs ranging from simple non-expensive analgesics to complex and expensive chemotherapeutic agents administered in the management of wound affect healing either positively or negatively (8). Attention should be directed towards discovering an agent, which will accelerate wound healing either when it is progressing normally (9), or when it is suppressed by various chemical agents. Aqueous extracts of *Chromolaena odorata* enhances haemostatic activity and stimulates granulation tissue and re-epithelization processes (10). Therefore, the plant can be of much therapeutic value in wound healing process which can be justified by doing the present study on the different solvent extracts of the leaves of *Chromolaena odorata* Linn.

MATERIALS AND METHODS

Plant Materials:

The fresh leaves from the plants of *Chromolaena odorata* were collected from hilly areas near Mohuda village in September'08. These were identified & authenticated by the taxonomist, Prof. (Dr.) S.K. Dash, H.O.D., PG Department of Bio-Science, College of Pharmaceutical Sciences, Mohuda, Berhampur, Ganjam (Orissa). The

specimen voucher of the leaf (Specimen Voucher No. CPS/DP-SV/10) was prepared and deposited in the herbarium of College of Pharmaceutical Sciences, Mohuda, Berhampur. The leaves were then thoroughly washed with purified water and completely shade dried. After proper drying, the leaves were powdered to 40 mesh size by using domestic grinder machine and then kept in well closed pet container with proper labeling.

Extracts from Plant Material:

The dried leaf powders of Chromolaena odorata were put into soxhlet extraction process using various solvents (Pet. Ether, Ethyl acetate & Methanol respectively) , which resulted in separation of constituents of different polarity ranges in different solvent extracts. The extractive values, colours and consistencies of the extracts were depicted in Table no.1.

Table No. 1: The colour, consistency and yield of different extracts of *Chromolaena odorata*

Sl. No.	Different Solvents	Colour of extracts	Consistency	Yield (%age) Extractive Value
1	Petroleum Ether Extract	Green	Sticky	5.7475
2	Ethyl acetate Extract	Black	Dry	10.7803
3	Methanol Extract	Brown	Sticky	8.8296

Determination of Ash values:

The different ash values like total ash, acid-insoluble ash, water soluble ash and sulphated ash were determined by using a little quantity of powdered crude drug and recorded in Table no.2.

Table No 2: Data showing ash values of leaves of *Chromolaena Odorata*

Sl. No.	Types of Ash	Yield (% w/w)
1	Total ash	9.1
2	Acid insoluble ash	0.3
3	Water soluble ash	3.7
4	Sulphated ash	0.6

Fluorescence characteristics of different extracts of *Chromolaena odorata* Leaves:

The different solvent extracts of leaf powder were exposed to UV light under a wavelength of 365nm and different characteristic colours were identified which were recorded in Table No.3. Again, the crude drugs were exposed to different chemical reagents and characteristic fluorescence were marked which were recorded in Table no.4.

Table No 3: Fluorescence characteristic of different extracts under UV Light at 365 nm.

Extracts	Fluorescence under UV Light (365nm)
Petroleum Ether Extract	Brick Red
Ethyl acetate Extract	Reddish brown
Methanol Extract	Green

Table No 4: Fluorescence characteristics of drug powder with different chemical reagents.

Sr. No.	Treatment	Through Naked eye	Fluorescence under UV light (365nm)
1	Untreated drug powder	Dark green	Dark green
2	Treated with 1N NaOH	Dark green	Dark green
3	Treated with 2N HCI	Deep greenish black	Green
4	Treated with 1N Nitric acid	Brown	Black
5	Treated with acetic acid	Green	Light green
6	Treated with Picric acid	Green	Black
7	Treated with Methanol	Green	Green with red
8	Treated with $FeCl_2$ solution	Greenish black	Black
9	Treated with 2 N H_2SO_4	Green	Yellowish brown
10	Treated with $BaCl_2$	Green	Green

Moisture content of the Crude drug:

The crude drug was introduced into the Karl-Fischer cell and Moisture content was determined by Merck Iyer Moisture balance, which was found to be 8.58%.

Qualitative phytochemical analysis of different extracts of the crude drug:

The pet.ether, ethyl acetate and methanol extracts obtained from the Soxhlet extraction and reflux condensation processes were analyzed for different phyto-constituents present in these by the method of qualitative phytochemical analysis. The specified chemical tests were carried out and the results were depicted in Table no.5.

Table No 5: Qualitative phytochemical analysis of different extracts of *C.odorata* leaves.

Sl. No.	Tests	Pet.Ether Ext.	Ethyl Acetate Ext.	Methanolic Ext.
1	Alkaloids	+ve	+ve	+ve
2	Carbohydrates	-ve	+ve	+ve
3	Cardiac Glycosides	-ve	-ve	+ve
4	Anthraquinone Glycosides	-ve	-ve	-ve
5	Gums & Mulilages	-ve	-ve	+ve
6	Proteins & Amino acids	-ve	-ve	-ve
7	Tannins & Phenolic Compounds	+ve	+ve	+ve
8	Triterpenoids	+ve	+ve	-ve
9	Steroids & Sterols	+ve	+ve	-ve
10	Saponins	-ve	-ve	-ve
11	Flavones & Flavonoids	+ve	+ve	-ve

EXPERIMENTAL DETAILS – ANTHELMINTIC ACTIVITY (7, 11, 12)

Drugs & Chemicals used as Standard and Control base:

Albendazole, Piperazine citrate & Normal saline solution.

Worm Collection & Authentication:

The anthelmintic activity was evaluated on adult Indian earthworm, "Pheretima posthuma" as it has anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. The said worms were collected from nearby areas of College of Pharmaceutical sciences, Mohuda, Berhampur in Sept'2009 and were authenticated from the Department of Pharmacology, CPS, Mohuda.

Anthelmintic activity study:

Eighteen groups of approximately equal sized Indian earthworms consisting of six earthworms in each group were released in to 50ml of desired formulation. Each group was treated with one of the following; vehicle (1% gum acacia in normal saline), piperazine citrate (15mg/ml), Albendazole (10 mg/ml) or extracts (2.5, 5, 10, 25 or 50 mg/ml). Observations were made for the time taken to paralyze and / or death of individual worms. Paralysis was said to occur when the worms do not revive even in normal saline. Death was concluded when the worms lose their motility followed with fading away of their body colour. The result was depicted in Table no 6.

Table No 6: Anthelmintic activity of leaf extracts of *Chromolaena Odorata*

Group	Treatment	Concetration used (Mg/ml)	Time taken for paralysis (min.)	Time taken for death (min.)
1	Vehicle	-	-	-
2	Piperazine citrate	10	18.50± 0.31	-
3	Albendazole	15	34.66± 0.72	63.83± 0.79
4	Pet-ether extract	2.5	37.815± 0.81	-
5	Pet-ether extract	5	24.841±1.85	33.08±3.098
6	Pet-ether extract	10	17.39± 0.539	28.453± 1.065
7	Pet-ether extract	25	16.788± 0.378	21.441± 1.100
8	Pet-ether extract	50	8.877± 0.310	18.11± 1.67
9	Methanol Extract	2.5	26.79± 1.33	-
10	Methanol Extract	5	18.83± 0.805	25.84± 0.776
11	Methanol Extract	10	15.56± 1.752	25.57± 0.6979
12	Methanol Extract	25	13.716± 0.596	19.23± 0.855
13	Methanol Extract	50	7.42± 0.589	14.588± 0.2733
14	Ethyl acetate	2.5	27.683± 0.811	-
15	Ethyl acetate	5	21.433± 0.854	27.783± 0.331
16	Ethyl acetate	10	17.65±1.075	25.866± 0.388
17	Ethyl acetate	25	16.58± 0.304	20.5±1.165
18	Ethyl acetate	50	8.11± 0.0813	14.9±0.199

EXPERIMENTAL DETAILS - WOUND HEALING ACTIVITY (5, 13)

Drugs used as Standard:

Neosporin ointment & Betadine ointment.

Experimental Animals Used:

Healthy Swiss Albino rats (150-180 gm) of both sexes were selected & obtained from the animal house of the College of Pharmaceutical Sciences, Berhampur. The rats were maintained at a well ventilated, temperature controlled (30°C) animal room for seven days prior to the beginning of experimental period. The animals were provided with normal food and water ad libitum. The rats were periodically weighed before and after the experiments. The rats were anaesthetized prior to infliction of the experimental wound. The surgical interventions were carried out under sterile conditions ketamine anesthesia (10mg/kg). Animals were closely observed for any infection. Those which showed any sign of infection, were separated and excluded from the study. This study was approved by the Animal Ethical Committee of C.P.S, Berhampur (Orissa).

Ointment formulation:

Formulation (5%, 7.5%, 10%, w/w)

Drug extract	-	500mg, 750mg, 1gm
Cetosteryl alcohol	-	1gm
P.E.G.6000	-	0.5gm
Petroleum jelly	-	7.5gm
Liquid paraffin	-	1 gm

Procedure:

1) Cetosteryl alchohol, PEG, pet. jelly & half of liquid paraffin were all mixed together and heated up to 100° C until it was melted.

2) Extract was mixed with the remaining half of liquid paraffin in a mortar until it became a paste.

3) Then the no.2 paste was added to no.1 mixture with constant stirring until it became a smooth mass.

Excision Wound Model: (14)

The rats were inflicted with excision wounds. The dorsal fur of the animal was shaved with an electric clipper and the area of the wound to be a created was outlined on the back of the animals with methylene blue using a circular stainless stencil. The skin of the dorsal thoracic region of rat was depilated and thoroughly cleaned then rat was anesthetized using ethyl ether and secured in normal vertebral column and 5cm away from ear using round real of 2 -2.5cm diameter wound area should be 450-500 mm^2 and 0.2 cm depth. Haemostasis was achieved by blotting the wound with cotton swab soaked with normal saline. Animal was placed in individual cages.

The group I animals were treated with simple solvent base (control). Group II & III, were treated with reference standards, Neosporin and Betadine ointments. Group IV V & VI were treated with 5%, 7.5% and 10% w/w of extract dissolved in solvents respectively for 14 days. The extract solutions (5, 7.5 & 10% w/w) at a quantity of 0.5 gm each were applied once daily to treat different group of animals. The simple solvent, Neosporin & Betadine ointments were applied in the same quantity to serve as control and standard respectively. Wound healing potential was monitored by wound contraction and wound closure time. The wound contraction was calculated as % reduction of wound area. The progressive change in wound area were monitored on polythene paper on post wounding days followed by 0^{th}, 4^{th}, 8^{th}, 12^{th}, 16^{th} till complete epithelization occurred. The results were noted in Table no.7.

Table 7: Wound healing activity of various leaf extracts of *Chromolaena odorata* & Excision wound model percentage of wound contraction post wounding days.

S. No.	Formulation	0th Day	4th Day	8th Day	12th Day	16th Day
1	Control base	454.83±0.703 (0)	391.17±1.601 (1.3)	389.52±1.18 (15.46)	284.26±1.18 (37.49)	152.7±0.74 (66.42)
2	Standard (Neosporin)	456.17±0.792 (0)	310.708±1.156 (31.88)	196.55±0.901 (56.91)	54.38±1.140 (88.08)	Healed (100)
3	Standard (Betadine)	456.34±0.856 (0)	330.708±1.38 (27.51)	209.15±1.0528 (54.24)	58.14±0.439 (87.24)	9.41±0.437 (97.95)
4	Pet ether Ext. 5%	455.67.±0.759 (0)	375.22±1.71 (18.56)	253.796±1.057 (44.22)	76.321±0.478 (82.98)	19.5±0.763 (95.71)
5	Pet ether Ext. 7.5%	456.5±0.428 (0)	349.22±1.43 (23.43)	218.466±1.592 (52.14)	62.6±1.117 (85.91)	14.18±0.600 (96.89)
6	Pet ether Ext. 10%	455.83.±0.763 (0)	343.49±1.152 (24.17)	213.85±1.2631 (53.16)	57.77±0.714 (87.38)	10.28±0.414 (98.14)
7	Ethyl acetate Ext. 5%	456.16±0.600 (0)	365.320±1.498 (20.86)	247.66±0.760 (46.7)	70.04±1.157 (85.73)	14.16±0.600 (98.14)
8	Ethyl acetate Ext. 7.5%	456.167±0.601 (0)	344.47±1.838 (25.45)	208.458±0.889 (49.19)	51.293±1.13 (91.52)	9±0.539 (98.78)
9	Ethyl acetate Ext. 10%	456.467±0.477 (0)	341.5±1.16 (26.22)	199.008±1.291 (51.16)	43.33.14±1.094 (96.3)	6.666±0.265 (100)
10	Methanolic Ext. 5%	456.5±0.428 (0)	361.32±0.874 (19.85)	243.12±0.965 (45.67)	64.125±1.14 (84.63)	8.433±0.550 (96.88)
11	Methanolic Ext. 7.5%	456.5±0.428 (0)	340.35±1.162 (24.5)	227.117±0.972 (54.30)	38.64±0.987 (88.75)	5.55±0.476 (98.02)
12	Methanolic Ext. 10%	457.0±0.365 (0)	336.3±1.370 (24.61)	223.127±0.963 (56.35)	16.82±0.69 (90.49)	Healed (100)

Values are expressed as mean± SEM; df = 5 n=6 animals in each group; Numbers in parenthesis indicate percentage of wound reaction: P< 0.001 when compared to control.

RESULTS AND DISCUSSION

The present investigation revealed that the methanol extract was endowed with potent anthelmintic property as compared to other extracts. The pet.ether and ethyl acetate extracts also possessed significant activity. The extracts were also found to be lethal to the worm's up to concentration of 10 mg/ml in pet.ether extract and up to 5 mg/ml in other two and paralysis occurred up to a concentration of 5 mg/ml in all the extracts. Potency of the extracts was found to be inversely proportional to the time taken for paralysis / death of the worms. The activities were comparable with the reference drugs, Piperazine citrate & Albendazole. The above findings justified the anthelmintic properties of the plant as suggested by the folklore practice.

The excision wound model test revealed that the methanolic extract was endowed with potent wound healing property as compared to other extracts. The

pet.ether and ethyl acetate extract also possessed significant activity. This was due to a numerous growth factors involved in the regulation of tissue repair and remodeling processes. The activity revealed concentration dependence nature of the different extracts. Potency of the extracts was found to be inversely proportional to the time taken for healing of wound. The activities were comparable with the reference drugs, Neosporin and Betadine. The above findings justified the wound healing properties of the plant.

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