ORIGINAL ARTICLE

Isolation and studies on chemotherapeutic potential of aloin

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Background: Plant-derived phytochemicals are gaining wide popularity owing to their diverse therapeutic potential and less side-effects. Aloin is one of the plant-derived flavonoids. Rutin has demonstrated cardioprotective, analgesic, and anticancer effects. **Aim:** The current work was focused to evaluate bioactive potential of aloin. **Materials and Methods:** Aloin was isolated from tobacco leaves. The structure was confirmed by H1 NMR spectroscopy. The isolated aloin was studied for possible antibacterial, antifungal, anthelmintic, larvicidal, and cytotoxic effects. **Statistical Analysis Used:** The results are expressed as mean ± standard error of the mean. Experiments were always performed in triplicates. Statistical comparison was performed using analysis of variance followed by Bonferroni's test (*$P < 0.05$). **Results and Conclusions:** Results of studies demonstrated that rutin effectively inhibited the growth of bacteria and fungi, as well as it also demonstrated the anthelmintic potential. There was a positive response for larvicidal and cytotoxic effects. These studies justify chemotherapeutic potential of aloin.

Key words: Aloin, antibacterial, antifungal, anthelmintic, larvicidal, cytotoxic

INTRODUCTION

Plants[1-4] and macrofungi[5-7] are considered to be an integral source of medicines and medicinal agents. *Aloe vera* is one of the important medicinal herbs known for medicinal and cosmetic use. Indian medicine considers its use for treatment of constipation, colic, skin diseases, worm infestation, and infections, whereas in Western society, it is recognized for its cosmeceutical potential.[8]

The aloin/barbaloin (10-beta-D-glucopyranosyl-1, 8-dihydroxy-3-hydroxymethyl-9 (is considered to be the most important phytoconstituents found in *Aloe* species. It is the C-glycoside of the aloe emodin anthrone. It is one of the important constituent found in all *Aloe* species.[9,10] *Aloe* is a potent source of antioxidant.[11] *Aloe* is known to demonstrate anti-obesity and weight lowering activity.[12-14] *Aloe* species are also reputed reduce radiation-induced oral mucositis[15] and oral submucous fibrosis.[16]

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It is also useful in retarding myocardial oxidative stress[17] hepatic periportal fibrosis[18] and hepatoprotective[19] potential. Topically, *Aloe* is proved to be effective in diaper dermatitis in children.[20] Apart from it, *A. vera* has demonstrated anti-inflammatory[21] and skin depigmenting effect.[22] An increase in immune response is also observed by use of *Aloe*.[23] It is also useful in periodontal diseases.[24] *Aloe*-emodin suppresses prostate cancer.[25] *Aloe barbadensis* restores lipid profile to normal in a letrozole-induced polycystic ovarian syndrome rat model.[26]

The current work was focused to isolate aloin from Aloes found in Mahakaushal region and to determine its chemotherapeutic potential.

MATERIALS AND METHODS

Test Organisms

Test Microbes

Bacterial and fungal cultures used in the present studies were obtained from Microbial Type Culture Collection (MTCC) IMTECH, Chandigarh. The bacterial strains were *Escherichia coil* MTCC 2960, *Pseudomonas auruginosssa* MTCC 4676, *Staphylococcus aureus* MTCC 3160, *Klebsiella oxytoca* MTCC 3030, *Bacillus subtilis* MTCC 1790, *Candida albicans* MTCC 183.

Test Worms

The roundworms (*Ascaridia galli*) were obtained from the intestine of freshly slaughtered fowls.

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Earthworms (*Pheretima phostuma*) (0.8–2 g) were collected from local gardens of Jabalpur, Madhya Pradesh, India.

Test Mosquitoes Larvae

The present study was conducted at Jabalpur (23.1700° N, 79.9500° E), Madhya Pradesh, India, during June-July, 2012. Larvae of *S. aegypti* were maintained at 25–30°C. The larvae were fed on a powdered mixture of dog biscuits and dried yeast powder at a ratio of 3:1. The adult colony was provided with 10% sucrose solution and 10% multivitamin syrup.

Cells for MTT Assay

The leukocytes were obtained from human peripheral blood, incremented with heparin and centrifuged with Phycoll is opaque (Histopaque-1077).

Chemicals

(3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethyl sulfoxide (DMSO) and standard sugars were obtained from Sigma (St. Louis, MO, USA). RPMI 1640 medium and fetal bovine serum (FBS) were the products of Gibco (Grand Island, USA). Clotrimazole and ampicillin were purchased from CDH, India. All other chemical reagents were analytical grade.

Isolation of Aloin

A total of 100 g of powdered *Aloe* was dissolved in 1000 ml of boiling water, stirred and filtered. The filtrate was acidified with sulfuric acid to precipitate resinous matter. Resinous material was removed by vacuum filtration and discarded. Filtrate was neutralized with sufficient quantity of ammonia solution and allowed to stand for few hours for slow crystallization of aloin. The crystals were separated, dissolved in 50% alcohol and recrystallized.[27] The structure of aloin was confirmed by ultraviolet-visible spectroscopy and H^1 NMR Spectroscopy.

Studies on Chemotherapeutic Potential

Antibacterial and Antifungal Studies

Aloin was dissolved in DMSO at a concentration of 1 mg/ml and used as working stocks. Ampicillin (25 µg) for bacteria, and clotrimazole (30 µg) for fungi were used as reference agents. Susceptibility test was determined by disc diffusion method.[28] The nutrient agar plates were prepared by pouring 15 ml of molten media into sterile petri plates. The plates were allowed to solidify for 5 min, 0.1% inoculum suspension was swabbed uniformly, and the inoculum was allowed to dry for 5 min. Aloin was loaded on 6 mm discs. The loaded discs were placed on the surface of medium and the extracts were allowed to diffuse for 5 min and the plates were kept for incubation at 37°C for 24 h for bacteria and 30°C for 48 h for fungi with yeast peptone dextrose agar. At the end of incubation, inhibition zones formed around the discs were measured with a transparent ruler in millimeters.

Determination of Minimal Inhibitory Concentration

A broth dilution susceptibility assay was used for the determination of the minimal inhibitory concentration (MIC).[29] Briefly, bacterial strains were cultured overnight at 37°C in nutrient agar; *C. albicans* was cultured overnight at 30°C in yeast peptone dextrose agar, respectively. Bacterial and fungal strains were suspended in their corresponding broths to give a final density of 106 and 105 organism/ml respectively. Dilutions of aloin 1, 2, 3 and 4 ranged from 10 µg/ml to 0.05 µg/ml were prepared in capped tubes. A control was also served; 20 µl from each of the test organisms was used to inoculate the tubes. The tubes were incubated at 37°C for 24 h for bacteria and at 30°C for 48 h for fungi. Tubes containing broth (2 ml) were inoculated with organisms and kept at 4°C in a refrigerator overnight to be used as standards. The MIC was recorded as the lowest concentration at which no microbial growth was observed.

Anthelmintic Assay

The anthelmintic assay was carried as per the method of Raghavamma and Rao,[30] with necessary modifications. Formulations (4 ml) containing different concentrations of aloin (25, 50 and 100 mg/ml) were prepared, and three worms (same type) were placed in it. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50°C).

Larvicidal Assay

The larvicidal bioassay followed the World Health Organization standard protocols[31] with slight modifications. Each of the concentrations of aloin (0.1–0.5%) was transferred into sterile glass petri dishes (9 cm diameter/150 ml capacity). Ten-third instar larval form of *S. aegypti* were separately introduced into different petri dishes containing graded concentrations, and the mortality were recorded after 24, 48 and 72 h of the exposure period. The data of mortality in 48 and 72 h were expressed by the addition of the mortality at 24 and 48 h, respectively. Dead larvae were identified when they failed to move after probing with a needle in the siphon or cervical region. The experiments were replicated 3 times and conducted under laboratory conditions at 25–30°C and 80–90% relative humidity.

Cytotoxic Studies (Colorimetric Mtt [Tetrazolium] Assay)

Cytotoxicity of aloin was measured as previously described by Mosmann.[32] The leukocytes were obtained from human peripheral blood, incremented with heparin and centrifuged with Phycoll is opaque (Histopaque - 1077). The cells were washed successively with RPMI 1640 medium and supplemented with 50 µM 2-mercaptoethanol and 5–10%

FBS, in a 5% CO_2 atmosphere. A volume of 100 µl of the solution was added to the cells in a ratio of 1 × 106 cells/well. These cells had been incubated for 4 h with different concentrations (0.5, 1.0 and 1.5 µg/ml) of aloin. Aloin and stock MTT solution (10 µl/100 µl medium RPMI) was added to all assay wells, and the plates were incubated at 37°C for 4 h. Acid-isopropanol (100 µl of 0.04 N HCl) was added to all the wells and mixed thoroughly to dissolve the dark blue crystals. The plates were read at 570 nm.

Statistical Analysis

The results are expressed as mean ± standard error of mean. Experiments were always performed in triplicates. Statistical comparison was performed using analysis of variance followed by Bonferroni's test (*$P < 0.05$).

RESULTS

Phytoanalytical Studies

Ultraviolet spectra of aloin showed λ_{max} at 360 nm, further structure of aloin was confirmed by H^1 NMR spectra [Figures 1 and 2].

Antimicrobial Studies

Among all the bacterial strains, maximum inhibition of growth was observed at *E. coli* and *K. oxytoca*. Growth of *C. albicans* was also inhibited. The minimum inhibitory concentration (in mm) was found to be 7 for *S. aureus*, 5 for *B. subtillis*, 7 for *E. coli*, 8 for *K. oxytoca*, 12 for *P. aurigenosa* and 14 for *C. albicans* [Tables 1 and 2].

Anthelmintic Assay

Following a brief stimulant effect on movement, earthworms and roundworms lost their movements, became paralyzed and finally died on exposure to extract. Formulation containing the aloin caused a dose-dependent paralysis causing loss of motility to loss of response to external stimuli, which ultimately progressed to death [Figure 3].

Larvicidal Assay

Results of the current work indicate that aloin in concentration 0.5% demonstrated lethal effect on larval motility and such effect was significantly ($P < 0.5$) then lower concentrations tested at 24, 48 and 72 h [Figure 4].

Cytotoxic Studies

The present study was undertaken to investigate the potential cytotoxic potential of *Pleurotus florida*. Our *in vitro* assay results showed that aloin was able to induce significant inhibition of cell growth in leukocytes within period of 24 h. Dose of 1.5 µg/ml effectively inhibited the growth of leucocytes and percent viability was 80.4 ± 1.8 [Figure 5].

DISCUSSION

The development of a new drug includes the use of a number of modus operandi ensuing in emergence of drug. A number of compounds are tested for pharmacological activities, and only some among them are therapeutically effective. As the process is time taking, thus use of *in vitro* techniques are found to be useful to screen out active pharmacological moiety which in turn reduces cost and time of the experiment.

The current work was focused to isolate aloin and to determine its chemotherapeutic potential. The results of

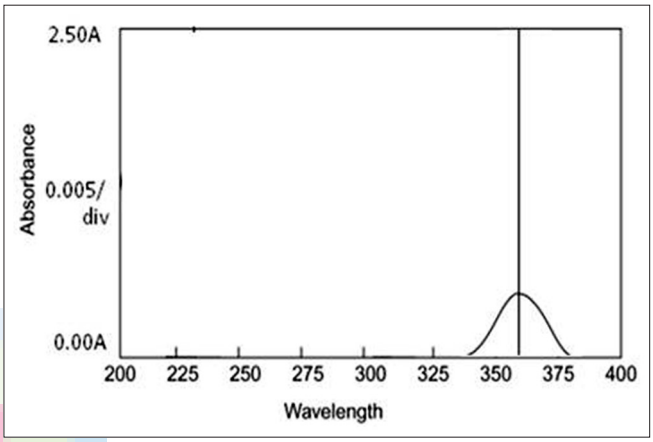

Figure 1: Ultraviolet spectra of aloin

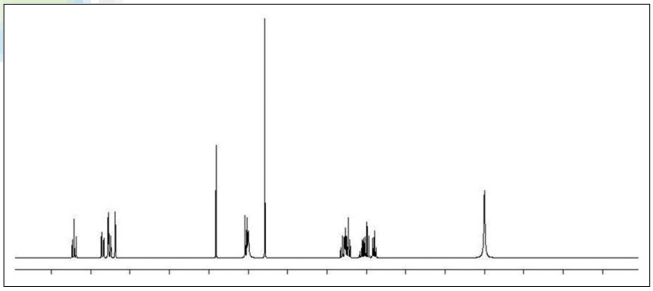

Figure 2: H1 NMR spectra of aloin

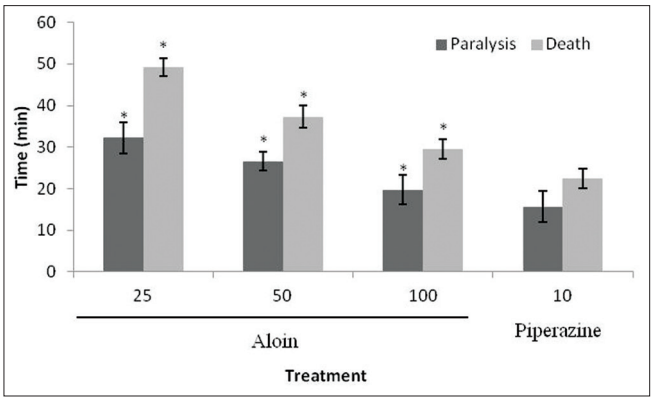

Figure 3: Anthelmintic activity of aloin each value is expressed as mean±standard deviation; (n=3); *P<0.001

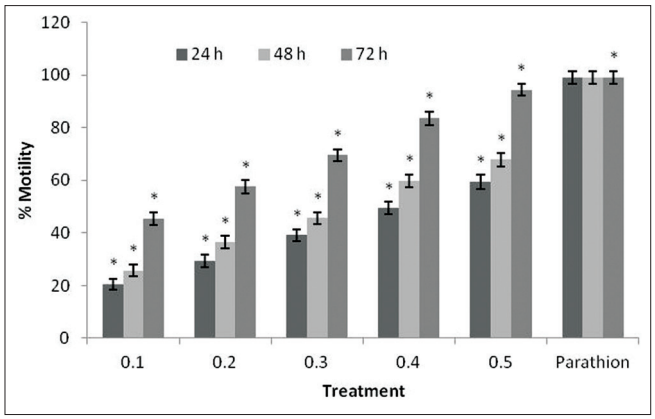

Figure 4: Larvicidal activity of aloin each value is expressed as mean± standard deviation; (*n*=3); **P*<0.001

Table 1: Antimicrobial activity of aloin and antibiotic sensitivity of microorganisms (zone size, mm)

Test organisms	Zone size (mm)		
	Aloin	Clotrimazole	Ampicillin
Staphylococcus aureus	14±1.5	-	22±1.2
Bacillus subtilis	6±0.8	-	9±0.8
Escherichia coli	10±1.2	-	9±1.3
Klebsiella oxytoca	12±1.1	-	5±0.3
Pseudomonas aurigenosa	13±0.8	-	9±0.7
Candida albicans	15±1.6	18±1.1	-

Each value is expressed as mean±SD. SD – Standard deviation

Table 2: Minimum inhibitory concentration of aloin of extract

Test organisms	MIC (mm)		
	Aloin	Clotrimazole	Ampicillin
Staphylococcus aureus	6	-	4
Bacillus subtilis	5	-	4
Escherichia coli	7	-	3.5
Klebsiella oxytoca	8	-	2.4
Pseudomonas aurigenosa	12	-	5.8
Candida albicans	14	9	-

MIC – Minimal inhibitory concentration

antimicrobial studies revealed that aloin is good antibacterial and antifulgal agent. Previous reports on *Aloe* sp. demonstrate such effect.[33-35] Anthelmintic studies on roundworm and earthworm also showed lethal effect of aloin on worms. This effect is comparable to standard piperazine. These results confirm the previous findings on *Aloe*.[36,37] Orally administered *A. vera* gel possess immunomodulatory activity.[38] Further, *Aloe* emodin has demonstrated apoptotic effect on human hepatoma Huh-7 cells by down-regulation of calpain-2 and ubiquitin-protein ligase E3A.[39] Schiff's base analogs of aloin also possess cytotoxic activity.[40] In the current work, cytotoxic potential of aloin on isolated leukocytes was observed, which in agreement with previous findings.

In nutshell, *in vitro* results demonstrate chemotherapeutic potential of Aloin. These primary results are encouraging,

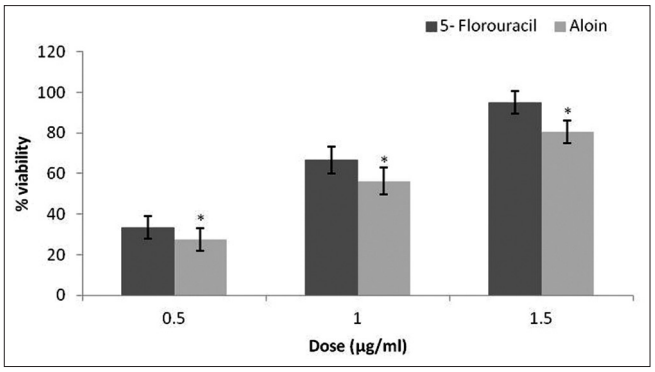

Figure 5: Effects of aloin on viability of human leucocytes. Each value is expressed as mean±standard deviation; (*n*=3); **P*<0.001

but a more systemic approach along with *in vivo* studies is required to explore the therapeutic potential of aloin in humans.

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