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*Full Length Research Paper*

# Isolation of lectin and lectin derivatives from *Haworthia limifolia*: An *in-vitro* investigation

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**The ability for medicinal plants to aid in wound healing lends itself to cell-agglutinating and sugar-specific protein. These are collectively termed lectins. Although, the function of these lectins in plants are limiting, the benefit to human in terms of wound healing is of vital importance. With many new infections arising as a result of bacteria, fungi and viruses developing immunity against western medicines, the world is now turning towards natural medicines as an alternative source for cures. *Harworthia limifolia* is currently used by indigenous people for sun burns, burns, sores as well as a systemic remedy and spiritual benefits. The population in the wild has drastically being reduced due to the pressures of harvesting by traditional healers for trade purposes. The current study investigates the presence of lectins or lectin like derivatives in *H. limifolia*. The derivatives were tested against rat, rabbit and human serum, and a positive reaction with human α-2-macroglobulin was observed. Furthermore anti-tumor and wound healing properties have been validated.**

**Key words:** *Haworthia limifolia*, lectins, agglutination, traditional use.

## INTRODUCTION

*Haworthia limifolia* is often used by traditional healers as a spiritual remedy as well as a treatment as blood purifiers and cures against coughs, skin rashes, sun burns, burns, etc. *H. limifolia* exhibits similar morphological characteristics as *Aloe* species and has a very close resemblance to *Aloe aristata.* Both *H. limifolia* and *A. aristata* are known by the Zulus as amatitibala.

The ability to cure diseases has been tested on many plant species by determination of their antimicrobial activities (Grierson and Afolayan, 1999; McGaw et al., 2000; Afolayan et al., 2002; Mathabe et al., 2006; Lategan et al., 2009). However, very little information is available on *Haworthia limifolia*, despite been extensively used for the treatment of a wide variety of ailments. In 2009, Fawole, et al., focused on its use in the treatment of gastro-intestinal ailments using test organisms that were directly or in-directly involved in gastro-intestinal disorders. Recently, the antimicrobial activity of *H. limifolia* was determined, the results of which proved to be valuable (Coopoosamy and Naidoo, 2011). In a recent study, Coopoosamy and Naidoo, 2011, investigated the traditional uses of *H. limifolia* in the treatment of sores, superficial burns, used as blood purifiers and to promote pregnancy in women and cattle. Furthermore, Mander, 1998, indicates the high demands of the plant where approximately 22,5 tons of the *H. limifolia* is traded in medicinal markets each year, comprising approximately 479 000 individual plants. Interestingly, the use of *H. limifolia* is mainly used by the Zulu speaking traditional healers and is mostly confined to KwaZulu-Natal. It has been shown that traditional healers from different localities use different medicinal plants for the treatment of common ailments (Mathabe et al., 2006). Ethno-pharmacological studies using plant extracts used by traditional healers from different localities and ethnicities have confirmed this

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(Coopoosamy and Naidoo, 2010; Lin et al., 2002; Ngobeli, 2002).

Lectins are glycoproteins that interact by binding to specific carbohydrates and are found in animals, plants and microorganisms (Jiang et al., 2010, Omega et al., 2008*).* Lectins-containing plants have been found in both monocotyledonous and dicotyledonous plant species including molds and lichens (Cheeke and Shull, 1983; Putsztai, 1991). However, they are most frequently detected in Leguminoseae and Euphorbiaceae occurring in various tissues within the same plant but at different cellular locations and with differing molecular properties (Cheeke and Shull, 1985; Putsztai, 1991). Studies have indicated the function of lectins in plants to be one of protein-protein interaction via lectin receptor kinases and RGD ligans (Chandra et al., 2006; Gouget et al., 2006).

Lectin containing plants would be essential for the medicinal world in assistance towards patients with extreme burns as well as severe injuries that needs to coagulate or clot blood to prevent further loss (Coopoosamy, 2010). Investigations have determined that lectin extracts, could produce similar agglutination activities even in a diluted form (Pusztai, 1991; Winters, 1993; Zhang et al., 2006). Investigations on laboratory animals being tested against lectins using oral administration have determined that the lectins interacted with the mucosa of the gastrointestinal tract resulting in acute gastrointestinal symptoms. The test animals seemed to have difficulty to survive and often death was observed (Yagi et al., 1985, Ogawara et al., 1985, Ogawara et al., 1987, Saito et al., 1989). When administered parenterally, the lectins can alter host resistance to infection or to tumor challenge and can even be highly allergenic under certain conditions (Yagi et al., 1985; Ogawara et al., 1985; Ogawara et al., 1987).

The ability of lectins to associate or make itself present in presence of tumor cell or surface infections have drawn interest for therapeutic purposes. Ogawara et al, 1985, has shown in their investigation that treatment with anti-lectin antibodies can suppress growth of tumor cells in agarose, and inhibit lung colonization *in-vivo*. This ability of lectin to promote binding of exogenous carbohydrate-containing molecules and further internalize them by endocytosis indicates a potential of lectins to be used in cancer treatment strategies (Fujita et al., 1978; Ogawara et al., 1987). Recent investigations, has been found that the respective hemagglutinins have binding specificities and their hemagglutinating activities are generally inhibited by monosaccharide or oligosaccharides (Fujita et al., 1978; Goldstein et al., 1980; Putsztai, 1991).

The presence of lectin-like substance in *Aloe* is well documented and reported as early as the eighteenth century (Goldstein et al., 1980, Imanishi, 1993, Saito, 1993, Suzuki et al., 1979); however, lectins have not previously being isolated from *H. limifolia*. In *Aloe* species*,* lectins are basically characterized by their cell aggluttination activities, particularly hemagglutination activities on sores (Coopoosamy, 2010; Goldstein et al., 1980; Imanishi, 1993; Saito, 1993) yet biological and pharmacological activities seem to differ among the respective lectins (Goldstein et al., 1980; Imanishi, 1993, Saito, 1993; Suzuki et al., 1979). To date characteristic activities and chemical properties of *H. limifolia* have not yet been sufficiently evaluated. Although lectins, which are found in abundance in many *Aloe* species no such isolation and investigations have been carried out in *H. limifolia*. This study attempted to investigate the presence of lectin and lectin derivatives in *H. limifolia* and the possible links of the use of *H. limifolia* by the traditional healers and indigenous people for treatment against sores, wounds, blood purifiers and rashes.

## MATERIALS AND METHODS

Plant material were collected from Silverglen Nature Reserve and transported to the laboratory in pots. A voucher specimen (SG2011/06) was prepared and stored in the Medicinal Plant Research Laboratory, Faculty of Natural Sciences, Mangosuthu University of Technology.

### Preparation of Aloctin A and Aloctin B from leaves of *H. limifolia*

The Aloctin A was prepared according to the method of Suzuki et al. (1979). *H. limifolia* whole plant (500 g) were crushed in a blender and then subjected to centrifugation to remove the coarse material. Solid ammonium sulphate was then added to obtain 40% saturation (40 g in 100 ml). The precipitate was collected by centrifugation, dissolved in 0.05 M carbonate-bicarbonate buffer (pH 9.5) and then re-centrifuged. 1 M Acetic acid was added to the supernatant to bring the pH to 4.4. The precipitate was then dissolved in enough 0.01 M phosphate buffer (pH 8.0) until completely dissolved and chromatographed on Sephadex G-200 column. A portion of each fraction was tested for detection of hemagglutinating and mitogenic activities with active fractions being pooled, condensed with Spectrapor membrane tube and rechromatographed on the same column. This active fraction was called Aloctin A. The acidic supernatant was lyophilized and dissolved in enough 0.05 M phosphate buffer (pH 8.0), until completely dissolved at a suitable concentration and then chromatographed on Sephadex G-100 column. This active fraction was called Aloctin B.

### Preparation for heamagglutinin and agglutination activity

One kilogram of whole plant of *H. limifolia* was homogenized using a blender and filtered through Whatman GF/A paper. The filtrate was dialyzed and concentrated, which was then lyophilized into powder form. The average yield was 497 mg. The lyophilized powder was used for immunodiffusion and immunoelectrophoresis studies.

### Preparation for wound healing

Fresh whole plant material (1 kg) was cut into small pieces and homogenized providing a mixture of exudates and particles. The mixture was then centrifuged at 4℃ and a greenish c oloured particle free liquid supernatant was collected (SI fraction). Pelleted material was re-centrifuged at high speed at a temperature of approximately 10℃. The high speed supernatant (SII f raction) and

pellet (HP fraction) were collected separately. SI and SII fractions were dialyzed and then concentrated at 4℃. A commerc ially stabilized *A. vera* gel was homogenized and separated into fractions as described above and used as control as it is known that *A. vera* gel does contain lectins. SI and SII fractions from this *Aloe* source were then concentrated and refrigerated at 4℃ together with all other *Aloe* fractions.

#### Ethanol extracts of *H. limifolia*

Fresh whole plant (1 kg) of *H. limifolia* was allowed to precipitate in absolute ethanol. The extract was then centrifuged to form pellets. These pellets were re-suspended in water and dialyzed against water. The extract was then lyophilized and reconstructed into a solution with water. Hemagglutinating activity was tested towards rat, rabbit and human erythrocytes. The extract was first dissolved in water and phosphate buffered saline (PBS) and then used for further dilution to avoid osmotic shock to the erythrocytes.

## RESULTS

The SI and SII fractions were found to exhibit hemagglutinating activity effective against rat erythrocytes. The concentrated SI fraction of *H. limifolia* contained markedly higher amounts of hemagglutination reactive substance than comparable fractions from commercially available control gel. The precipitation tests carried between rat sera and concentrated SI fractions from *H. limifolia* reacted more positively to human sera as compared to rat sera. These results is in line as work of Winters et al. (1981), where the investigation of extracts from three *Aloe* species did not react in the precipitation test to canine sera from normal and tumor bearing adult dogs. Concentrated SII from *H. limifolia* did not show immunoprecipitation reaction for any other sera (Tables 1 and 2).

Indomethacin, prednisolone carrageenin, liquid paraffin and heated killed *Mycobacterium butyricum* were used on test rats. Induction of arthritic syndrome in laboratory rats into the interplantar surface of the right hind foot was achieved by an intradermal injection liquid paraffin (0.1 ml) containing 0.6 mg of heat killed *Mycobacterium*. Administrations of test compounds were done either orally or intraperitoneally each day for 15 days beginning a day prior to the injection (Table 3).

Edema was induced in the hind paw of the rats by a sub-cutaneous injecting 1% Carrageenin solution (0.05 ml in 0.9% NaCl) (Table 4). Aloctin A was suspended in 0.9% NaCl and administered intraperitoneally 30 minutes prior to the injection.

Volume measurements (amount of water displaced) were made immediately prior to and at one, three, four, and five hours after injection (Table 4). These volume measurements were determined by the water displacement method. The effects of the compounds were expressed in terms of percent inhibition in the swelling volume of the control animal versus the treated animals. Aloctin A and Aloctin B in *H. limifolia* agglutinate erythrocytes of the test species (Table 5). The agglutinating activity by Aloctin A and Aloctin B was increased proportionally when 0.1% Trypsin was added to the treatment of rat serum as compared with that of un-treated erythrocytes. Aloctin B had a stronger hemagglutinating activity compared to that of Aloctin A but Aloctin B had no mitogenic effect on human erythrocytes. The human serum proteins that reacted to crude extract of control gel were identified as α-2-macroglobulin and α1-antitrypsin by immunoelectrophoresis (Fujita et al., 1978). When Aloctin A from *H. limifolia* was tested against rat, rabbit and human serum, a positive reaction with human α-2-macroglobulin was observed.

Superficial administration of Aloctin A of *H. limifolia* to the infected area effectively suppressed the swelling in the injured foot and on the inflamed lesion. Oral administration did not affect adjuvant arthritis. However, hind paw edema was induced by subcutaneous injection of carrageenin solution into the hind foot pads of rats. Administration of Aloctin A markedly inhibited carrageenin induced edema (Tables 4 and 5).

The results clearly indicate that Aloctin A at all dose levels effectively suppresses the swelling of adjuvant arthritis, with the optimal dose of 5 mg/kg/day. The activity of Aloctin A given intraperitoneally appeared to be higher than that of indomethacin given orally, and was nearly equal to prednisolone given orally. During the course of this experiment no side reactions of Aloctin A were noted.

## DISCUSSION

This investigation has provided insight that the constituents of *H. limifolia* extracts that reacted with α-2-macroglobullin and α1-antitrypsin were lectins or lectin derivatives. These lectin derivatives are implicated in anti-inflammatory action and their therapeutic effect on burns, as serum proteins reacting to the extract were α-2-macroglobullin and α1-antitrypsin, which are known to be the most representative protease inhibitors. The Secondary use of *H. limifolia* for the treatment of inflammation of joints has been noted from the results obtained. This results, hence, justifies the use of *H. limifolia* by traditional healers for the wide application, including inflammation and wound healing.

The positive results obtained in response to hemagglutinating activities of *H. limifolia* further substantiates the use of this species in the treatment of various ailments, such as wound healing, blood coagulation and as a skin-soothing agent. The precipitate forming reactivity with serum proteins, for example α-2-macroglobulin contributes to use on effects of the immune system. The properties of all these positive responses of lectins with regard to hemagglutinating activities are well-documented in literature (Coopoosamy,

**Table 1.** Anti-tumor activity of aloctin A against sarcoma meth A (Ascites Form) in laboratory mice.

| Treatment | Dose (mg/kg/day × days) | Average TPCV[a] (ml) | T/C ratio (%) | Complete Inhibition (%) |
|---|---|---|---|---|
| Control | | *0.64 | | 0 |
| Aloctin A | 10 × 5 | 0.05 | 7.8 | 67 |
| Aloctin A | 0.2 × 5 | 0.45 | 70.3 | 17 |
| Aloctin A | 0.4 × 5 | 0.41 | 64.1 | 17 |

[a]Total packed cell volume, *p < 0.001, significantly different from control. T/C % = TPCV of treated group / TPCV of control X 100. T = Treated group, C = Control, A total of 10 mice were used for each treatment. The average total packed cell volume is indicated.

**Table 2.** Anti-tumor activity of aloctin A against P388 in $CDF_1$ mice.

| Treatment | Dose (mg/kg/day) | No. of Mice | Survival time range (days) | Time M.S.T. (days) | T/C (%) |
|---|---|---|---|---|---|
| Control | | 10 | 8 – 9 | 8.50 | |
| Aloctin A | 10 | 6 | 9 – 11 | 9.98 ** | 117.4 |
| Aloctin A | 5 | 5 | 9 – 11 | 9.30* | 109.4 |
| Aloctin A | 1 | 6 | 8 – 10 | 9.06 | 106.6 |
| Aloctin A | 0.2 | 6 | 8 – 10 | 8.63 | 101.5 |

M. S. T., Median Survival Time, T/C % = M. S. T. of treated group / M. S. T. of Control × 100. Evaluation of anti-tumor activity; T/C % in life-span *, ** significantly different from control *P < 0.01, ** P < 0.001. Dose was calculated as: mg of test agent / mass in kg of mice/ duration in days. T = treatment C = control TC % = TPCV of treated group/ TPCV of untreated group × 100. n = 6.

**Table 3.** Aloctin A, Indomethacin and Prednisolone effect on Arthritis Induced in Rats by Mycobacterial Adjuvant. n = 6.

| Compound | Daily dose (mg/kg) | Route | B.W. Gain day 0 – 21 | Inhibition injected day 14 | (%) Hind paw day 21 | Inhibition secondary day 14 | (%) Lesion day 21 |
|---|---|---|---|---|---|---|---|
| | 0.5 | i.p | 14 ± 10 | 14.4 | 9.3 | 27.4 | 16.4 |
| | 2.5 | i.p. | 36 ± 9 | 39.3 | 28.0 | 23.6 | 29.3 |
| Aloctin A | 5.0 | i.p. | 43 ± 9 | 57.2 | 55.4 | 43.7 | 74.4 |
| | 7.5 | i.p. | 6 ± 1 | 49.4 | 29.3 | 36.3 | -2.7 |
| | 10 | i.p. | 5 ± 1 | 22.6 | 4.4 | 28.6 | -5.6 |
| Indomethacin | 2.0 | p.o. | 10 ± 6 | 38.1 | 24.9 | 13.1 | -1.8 |
| Prednisolone | 2.0 | p.o. | 0 ± 1 | 58.3 | 41.2 | 83.1 | 37.3 |
| Control | | | 10 ± 8 | 0 | 0 | 0 | 0 |

A total of 10 mice were used for each treatment. The average total packed cell volume is indicated. Each value of Inhibition (%) is average of six rats per group. B. W = Body weight gained (in grams). * Significantly different from control *P < 0.001.

2010; Imanishi and Suzuki, 1984; Imanishi, 1986; Imanishi, 1993; Saito, 1993). The hemagglutination activity is attributed to the presence of Aloctin A and Aloctin B of *H. limifolia* which was isolated and used in test to substantiate its presence. The ability to coagulate at a wounded site and form a barrier against blood loss would assist in alternative treatments against wound healing and in arthritic patients. Furthermore, the ability to act against inflammation and swelling contributes to the plants anti-inflammatory properties. Table 4 indicates that aloctin A shows a 95.2% inhibition at a dose of 10 mg/ml and 55.9% inhibition at a dose of 1 mg/ml after 5 h. Indomethacin provided effective results, 58.9%, at a dose of 2 mg/ml after 5 h. This indicates that the extract from *H. limifolia* has a higher potential of wound healing as compared to traditional western medicine.

Strong hemagglutinating properties were found from the ethanol extract of *Aloe* species were described to have lectin like properties, that is, strong hemagglutinating effects towards human erythrocytes (Bouther et al., 1996). *H. limifolia* ethanol extract has shown equal hemagglutinating properties against all serum types

**Table 4.** Aloctin A and indomethacin effect on swelling of rat hind paw induced by carrageenin n = 6.

| Compound | Dose (mg/kg) | Route | % Inhibition (hour) 1 3 4 5 | | | |
|---|---|---|---|---|---|---|
| | 0.5 | i.p. | 10.0 | 31.3 | 37.1 | 26.6 |
| | 1.0 | i.p. | 10.4 | 63.9 | 63.0 | 55.9 |
| Aloctin A | 5.0 | i.p. | 12.8 | 51.4 | 55.9 | 58.7 |
| | 7.5 | i.p. | 5.0 | 90.2 | 87.8 | 95.1 |
| | 10.0 | i.p. | 2.1 | 93.7 | 94.4 | 95.2 |
| | 0.5 | p.o. | -14.4 | 18.9 | 19.8 | 30.4 |
| Indomethacin | 1.0 | p.o. | -28.3 | 31.9 | 41.6 | 41.1 |
| | 2.0 | p.o. | 13.5 | 56.0 | 63.8 | 58.9 |

Each value is a mean of six mice per group. i.p. = intraperitoneally, p.o. = orally.

**Table 5.** Hemagglutinating activity of *H. limifolia* against various serum types.

| Compound | Activity of serum type | | | | |
|---|---|---|---|---|---|
| | Rat | Rabbit | Human | Rat + 1% Trypsin | Human + 1% Trypsin |
| S I Fraction | + | + | ++ | ++ | +++ |
| S II Fraction | ++ | + | ++ | ++ | +++ |
| Ethanol extract | +++ | ++ | +++ | +++ | +++ |
| Control (*Aloe vera*) | +++ | ++ | +++ | +++ | +++ |

+ = Low activity, ++ = Medium activity, +++ = High activity. Control = Commercially obtained *A. vera* extract.

under investigation. The positive results implied that *H. limifolia* has properties which deal with anti-inflammation and anti-arthritis as investigated on *Aloe* species (Saito et al., 1982, Imanishi and Suzuki, 1984).

## Conclusion

The results of the *in-vitro* testing of *H. limifolia* extracts provided suitable evidence to substantiate the use of this plant by traditional healers for various treatments, such as burns, sores, and blood cleansers. Furthermore, due to the exploitation of this species in the wild, a suitable rehabilitation programme should be established to maintain the current population as well as a re-introductory to the wild.

This would enable this important traditionally utilize species to not reach an extinct status. Knowledge transfer to indigenous healers would be contributory to the understanding of sustainable harvesting and conservation of this species. Furthermore, with the ability for the plant extracts to be effective as a blood coagulant and as a blood purifier, there is potential that the extracts could be effective against HIV/AIDS related ailments.

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