

# Evaluation of wound healing properties of methanolic extract of *Ageratum houstonianum*-Mill.

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## Abstract

In the present study methanolic extract of the *Ageratum houstonianum* Mill. leaves has been investigated for its phytochemical composition and wound healing property. Phytochemical screening revealed the presence of steroids, alkaloids, tannins, glycosides, diterpene, flavonoids, saponins and triterpenes in the extract. Presence of flavonoids indicated the antioxidant property of the extract which can be contributed to its wound healing property. This hypothesis led to the wound healing studies on excision wound models of rat. Rate of wound constriction have not shown much difference when compared to the control group. Histopathological examination of the scar tissue showed reduction in the infiltration of inflammatory cells in a dose dependant manner when compared to normal control group. So it can be concluded that the wound healing property of the extract may be due to the antioxidant and antiseptic property of the extract.

**Keywords:** *Ageratum houstonianum* Mill; Wound healing; Phytochemicals; Histopathology

## Introduction

Medicinal plants have been employed in folk medicine from the time immemorial for various ailments like atherosclerosis, anti-inflammatory, anti-cancerous and antimicrobial properties (Kumar *et al*, 2007). These plants are least documented and scientifically evaluated for their properties by the modern medicine. *Ageratum houstonianum* Mill. is one such plant which has been studied least and it belongs to the family Asteraceae. The genus *Ageratum* consists of approximately 30 species but only few species have been phytochemically investigated. *Ageratum houstonianum* Mill, a native to Mexico, Central America and the Caribbean is prone to becoming a rampant environmental weed when grown outside its natural range (Narendra *et al*, 2014). *Ageratum houstonianum* Mill. and its related species *Ageratum conyzoides* are well known for its medicinal properties (Musthafa *et al*, 2005). It is available in the forest regions of Kerala especially in Shola forests. It is commonly known as Ammanpathri among local tribes. Recently *Ageratum houstonianum* Mill. has been screened for its antimicrobial and mosquitocidal activities (Tennyson *et al*, 2011). The present investigation aims at the estimation of phytochemicals present in the methanolic extract of *Ageratum houstonianum* Mill. leaves and its wound healing properties.

## Materials and Methods

### Collection of plant and Methanolic extraction of *A. houstonianum* Mill. leaves

Fresh leaves of *Ageratum houstonianum* Mill. was collected from Idukki district of Kerala, shade dried and powdered coarsely in a temperature controlled plant sample pulveriser. Powdered leaves (500g) were extracted with methanol in soxhlet extractor at room temperature. The crude methanolic extract of *Ageratum houstonianum* Mill. was used for the study. Methanol was removed from the extract using rotary vacuum evaporator and then dried completely by keeping at room temperature to use for further experiment.

### Phytochemical Screening

Methanolic extract of *Ageratum houstonianum* Mill. leaves was tested qualitatively for the presence of various phytochemical constituents namely steroids, alkaloids, tannins, flavonoids, glycosides, diterpenes, triterpenes, saponins and cardiac glycosides as per the standard protocols (Harbone, 1991).

### Wound healing studies

#### Preparation of ointment

*Ageratum houstonianum* Mill. leaves collected locally, shade dried, ground and its alcoholic (methanolic) extract was prepared. Ointment with two different concentrations of plant extract in soft paraffin was then (10% and 20%) prepared and stored.

#### Animals Used

Healthy adult male Wistar rats (120-150gm) were procured from small animal breeding Station College of Veterinary and Animal Sciences, Mannuthy, Thrissur. The animals were housed in polypropylene cages with free access to standard feed and water. Animals were housed for a week, as an acclimatization period before the commencement of the experiment. The experimental protocols involved in this study were approved by the Institutional Animal Ethics Committee, College of Veterinary and Animal Sciences, Mannuthy, Thrissur.

| Groups    | Drug used                | Number of animals used |
|-----------|--------------------------|------------------------|
| Group I   | Plant extract 10%        | 6                      |
| Group II  | Plant extract 20%        | 6                      |
| Group III | Control (Normal Healing) | 6                      |



Fig. 1 & 2. *Ageratum houstonianum* Mill. and its leaf

### Excision Wound model

The rats weighing around 120- 140g were anaesthetized by an intra peritoneal injection of xylazine and ketamine. The anaesthetised animals were kept in ventrodorsal position and the thoraco dorsal part was shaved properly. Then the area was sterilized and a square shaped full thickness cutaneous wound of skin ~ 1x1 cm<sup>2</sup> was created and the area was left undressed. These animals were then divided into groups. Ointment was applied twice daily on treatment groups.

*Tissue harvesting*

Three animals from each group were sacrificed on days 4<sup>th</sup> and 8<sup>th</sup> with thiopentone sodium injection and the granulation/healing tissue was carefully excised out and was immediately preserved in 10% neutral buffer formalin for histopathological observation (Haematoxylin and Eosin staining).

## Results and Discussion

The results of phytochemical screening of Methanolic extract of *A. houstonianum* Mill. extract are presented in Table 1. Phytochemical screening revealed the presence of steroids, alkaloids, tannins, glycosides, diterpene, flavonoids, saponins and triterpenes in the extract of *A. houstonianum* Mill. The phytochemical constituents were similar to the earlier findings by Quijano *et al*, 1982.

**Table 1.** Phytochemical screening of methanolic extract of *A. houstonianum* Mill. extract

| Phytochemicals     | Tests   | Methanolic extract of <i>A. houstonianum</i> |
|--------------------|---|--|
| Steroids           | Salkowski test  | +ve  |
| Alkaloids          | Mayer's test<br>Wagner's test, Hager's test, Dragendroff's test | -ve<br>+ve                                   |
| Phenolic compounds | Test for Phenolic compounds                                     | -ve  |
| Tannins            | Ferric chloride test<br>Gelatin test                            | +ve<br>-ve                                   |
| Glycosides         | Sodium hydroxide test<br>Benedict's test                        | +ve<br>-ve                                   |
| Diterpene          | Test for diterpenes   | +ve  |
| Triterpenes        | Salkowski test, Liberman burchard test                          | +ve  |
| Saponins           | Foam test   | +ve  |
| Cardiac glycosides | Keller kilanis test   | -ve  |
| Flavonoids         | Ferric chloride test, Lead acetate test                         | +ve  |

*Wound healing studies*

Grossly, both *A. houstonianum* Mill. treated group and the control group (undergone normal healing) showed considerable signs of dermal healing. Group treated with *A. houstonianum* mill. healed faster with considerable reduction in wound width when compared to the group received the control treatment. Histopathology of the scar tissue of the rats treated with 10% and 20% ointment showed reduction in infiltration of inflammatory cells in a dose dependent manner shown in Figure 1 and Figure 2. This may be due to the presence of naturally occurring antioxidant molecule flavonoids which can scavenge several free radicals which could be the major contributing factor for the wound healing property of the extract. It has been reported that the antioxidants plays a significant role in the wound healing process and significantly improve wound healing and protect tissues from oxidative damage (Shukla *et al*, 1999).

Present study could identify that the reduction in inflammatory cells and quality of healing has not shown much difference when compared to the control group even though there was differences on gross examination of the wound. So it can be speculated that the healing property of the plant is mainly due its antiseptic and antioxidant properties as reported (Narendra, 2014).

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