



## Antibacterial Activity of Aqueous Extract and the Boiled Steam of *Atalantia Ceylanica* Leaves

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### ABSTRACT

The in vitro antibacterial activity of *Atalantia ceylanica* (arn.) oliver. leaves were tested qualitatively against selected four bacterial strains with the agar dilution method at the Gampaha Wickramarachchi Ayurveda University, Gampaha, Sri Lanka. Medicinal flora is used heavily now a day to treat various diseases. Nevertheless, the scientific studies to find their pharmacological values is limited. Thus, the study was arranged to fill the gap to a certain extent to harness natural resources effectively in health. Inhalation of the steam of boiled leaves of *Atalantia ceylanica* is used commonly by villagers to cure respiratory ailment such as influenza. Etiologies of influenza could be bacterial or viral. The study was setup to screen the antibacterial properties of *Atalantia* leaves. Leaves of *Atalantia* were boiled and both steam as well as aqueous extract was used directly and separately with Mueller-Hinton agar to prepare the plates. Possible respiratory pathogens such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and Methicillin resistant *Staphylococcus aureus* were inoculated separately into the pates which contain the neat concentration of phytochemicals. Bacteria were spread, and plates were incubated. For the control, the plates which were prepared with distilled water and Mueller-Hinton agar in place of leaf extract were used. Even after the duplicated experiment, no strain was found to be inhibited in the test plates, which indicate that the neat concentration of the raw chemical compound of particular leaves has no inhibitory action on tested strains. As the respiratory infection is not only caused by bacteria but also by viruses, perhaps phytochemicals might have possessed antiviral as well as antibacterial properties on organisms other than the tested strains. Occasionally, the chemicals of the particular plant leaves may contain anti-inflammatory properties, which cure the respiratory symptoms.

**Key words:** *Atalantia ceylanica*, Bacteria, Antibacterial effect, Leaves, Phytochemicals,

### Introduction

Usage of natural herb as a remedy for most illnesses, has become a trend in the modern society.

This could be due to the facts of financial burden, drug allergy as well as drug resistant. During the period of corona pandemic, certain natural medicinal flora was used for steam inhalation as they were easy to collect and prepare<sup>(1)</sup>. The *Atalantia ceylanica* is one such.



Figure 1: a picture of *Atalantia ceylanica* plant <sup>(1)</sup>

Inhalation of steam of boiled leaves of *Atalantia* was utilized as a remedy for respiratory tract illnesses such as influenza by the ancestors since long ago. Even in the traditional medicine, and Ayurveda *Atalantia* is used for many diseases such as for treating fatty liver, catarrh, arthritis.

The plant grows up to three meters and commonly found in Sri Lanka and South India. It is in the family of Rutacea<sup>(1)</sup>.

Though, several antioxidant studies have been done on the plant, studies on antibacterial effects of the plant are few in number. The current study aim on the detecting antibacterial effect of watery extract of *Atalantia* leaves and to fill the gap of less studies done on the plant. This could be a good platform for scientist to do more and more researches on the plant.

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## Methods

The test followed the normal routine procedure that is followed by villagers to inhale the plant steam.

Fresh fifteen (15) mature, normal size leaves of the *Atalantia ceylanica* plant were collected and washed to clean thoroughly.

The leaves were placed in a 2 L of round bottom flask and mixed up with 500 ml of distilled water.

The mixture was boiled (100 °C) for ten (10) minutes.

The steam was cooled with a condenser fixed to the flask and the steam was collected in a receiving flask.

The aqueous extract in the round bottom flask was cooled and filtered with a gauze filter.

The filtrate of aqueous extract (neat concentration) and the cooled steam were separately used to prepare the culture plates (plant-based culture media) according to the agar dilution method <sup>(2)</sup> as follows.

The neat aqueous extract, 160ml was mixed up with 6.08 g Mueller hinton agar (MHA).

Then the pH of the mixture was adjusted to 7.4 with Potassium Hydroxide (KOH).

The mixture was sterilized with steam at atmospheric pressure method (100 °C, 1 atm, 90 min). The method was used to reduce the destruction of phytochemicals.

Total eight (08) culture plates were prepared with melted MHA which contain a neat concentration of plant extract.

The plant extract was replaced by distilled water in the preparation of control plates.

To test the effect of distillate, the plates were prepared with distillate according to same procedure (Control plates were prepared with the distilled water in place of distillate).

Stock cultures of bacterial strains of *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and Methicillin resistant *Staphylococcus aureus* (MRSA) were maintained in the laboratory

Two pure colonies of each bacterial strains were sub cultured in 5 ml of nutrient broth and incubated at 37°C for four hours (04 hrs.) to prepare broth cultures.

Then each broth culture was adjusted to 0.5 Mc far land standard and 10 microliters of each was inoculated separately to the plates prepared previously.

The number of total four plates were streaked out and incubated at 37°C overnight.

The same procedure was adopted for the set of control plates.

The test was duplicated.

The same procedure was carried out to screen the antibacterial effect of distillate of the plant.

(The respective control test was carried out with the culture plates prepared with distilled water in place of distillate).

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## Result and Discussion

Table 1- The result of aqueous extract of *Atalantia ceylanica*

Bacterial strain	Result	
	Test	Control
<i>Pseudomonas aeruginosa</i>	Positive growth	Positive growth
<i>Escherichia coli</i>	Positive growth	Positive growth
<i>Staphylococcus aureus</i>	Positive growth	Positive growth
<i>Staphylococcus aureus</i> (MRSA)	Positive growth	Positive growth

Table 2- The result of distillate of *Atalantia ceylanica*

Bacterial strain	Result	
	Test	Control
<i>Pseudomonas aeruginosa</i>	Positive growth	Positive growth
<i>Escherichia coli</i>	Positive growth	Positive growth
<i>Staphylococcus aureus</i>	Positive growth	Positive growth
<i>Staphylococcus aureus</i> (MRSA)	Positive growth	Positive growth

According to the result it is obvious that there is no antibacterial effect of both distillate and aqueous/ watery, extract of *Atalantia* for the tested four strains of bacteria.

The steam was condensed, cooled and was used to prepare culture plates as it is. Thus, if the steam contains water soluble phytochemicals, they may persist in the neat concentration. Villagers also use the steam which contains the neat concentration of phytochemicals of *Atalantia*. The watery extract also contains the neat concentration of water-soluble phytochemicals.

Though, Influenza (flu) is caused by both bacteria and viruses, viruses are more common in this regard. The current study has screened the antibacterial nature of *Atalantia*. Though, the bacterial strains were resistant to the plant materials, it could have possessed some phytochemicals, which react against viruses (antiviral compounds). This may be the secret behind long-lasting usage of the plant for respiratory ailments.

Further, the inhalation of pant steam may relieve respiratory congestion and discomfort. This could be due to the anti-inflammatory properties of the phytochemicals of the plant which was not tested in the study, nevertheless in the other studies.

Anyway, in the study carried out by karunathilaka *etal* in 2022<sup>(3)</sup>, with methanol extract, found to have antibacterial properties of the relevant plant leaves. This study revealed that *Escherichia coli*, and methicillin-resistant *Staphylococcus aureus* were sensitive to the methanol extract of plant leaves. According to the finding it can be mentioned that the *Atalantia* leaves may contain certain volatile, lipid /alcohol soluble phytochemicals which are with antibacterial properties. This fact is supported by the study carried by Dharmadasa *etal*, in 2020<sup>(4)</sup>. They have isolated five volatile phytochemicals from the particular plant leaves. Anyway, compared to other species of the Rutacea family, *Atalantia ceylanica* has shown a few volatile indigents. As the current study has been based on aqueous medium, water-soluble antibacterial phytochemicals, might not be positive in the plant.

However, in the study carried out by Bandara *etal* 1990<sup>(5)</sup> also found to have no remarkable antibacterial activity of *Atalantia* plants. The extract they also used was the condensed steam of the boiled plant leaves which is similar to current study.

The study carried by Fernando *etal* in 2014<sup>(6)</sup>, found to have antioxidant properties of the watery leaf extract of *Atalantia*. This antioxidant property may also assist in reducing the oxidative stress which aroused in respiratory ailment, hence to minimize the inflammation and its sign and symptoms. In the study carried in 2020 by Jayasinghe *etal*<sup>(7)</sup>, revealed that there is an antioxidant property in *Atalantia ceylanica* leaves. Possessing of this property by the plant has been proved by several other invitro studies as well <sup>(8)(9)(10)</sup>.

## Conclusion

All the tested bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and Methicillin resistant *Staphylococcus aureus*) were resistant to the neat watery extract of tested plant. Perhaps, *Atalantia* may contain phytochemicals which act as antiviral agents (in most of the cases influenza is caused by viruses). Further, it may contain ingredients which reduce inflammation also. These two facts could be the reason for usage of *Atalantia* by ancestors for such longtime for respiratory issues.

## Conflict of interest

It's to mention that as the authors of the research study (Antibacterial activity of aqueous extract and the boiled steam of *Atalantia ceylanica* leaves), we have no conflict of interest on the study.

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