

IN VITRO ANTILEPTOSPIRAL ACTIVITY OF  
*PLECTRANTHUS AMBOINICUS (LOUR) SPRENG*

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Summary

An in-vitro anti leptospiral activity of the aqueous extract and volatile oil from the leaves of *Plectranthus amboinicus (Lour) Spreng* family; *Lamiaceae* was conducted on laboratory cultured Leptospire. (*L.biflexa*) and compared with standard drug amoxycillin. The results showed both aqueous extract and volatile oil was effective in the concentration of 500 mcg /100µl and 50 mcg/ 100 µl respectively. It was already proved that the plant *P.amboinicus* has antimicrobial and antifungal activity. Further investigation in the isolation and purification of the active principle responsible for the activity may provide an antimicrobial, antifungal along with antileptospiral activity drug template from natural source.

Keywords

Leptospirosis, *Plectranthus amboinicus*, *Coleus amboinicus*, *Coleus aromaticus*, antileptospiral activity.

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### Introduction

Leptospirosis, a zoonosis is an acute febrile illness caused by microorganisms of the genus leptospira. Leptospire are bacteria infecting large variety of domestic and wild animals which excrete them in their urine. Man becomes infected when he comes into contact with an infected animal either directly or more frequently indirectly through environmental contamination with infected urine. The genus Leptospira comprises thin, tightly coiled spirochaetes which are characterized by very active motility, both rotating and bending. Usually one or both ends of the cell are bent or hooked. <sup>(1)</sup>

The initial symptoms of acute leptospirosis are the symptoms of an acute febrile illness- such as malaise, depression, anorexia, conjunctivitis accompanied by fever. If the disease progresses, symptoms and signs appear which are more characteristic of leptospirosis, such as bleeding and its consequences like jaundice, central nervous system involvement, liver and renal failure. <sup>(1)</sup> Leptospirosis is typically a self-limiting disease, but it can have significant morbidity and mortality if not treated or prevented. Leptospirosis has recently been described to cause concomitant infection with malaria which leads to therapeutic dilemma among febrile patients. <sup>(2)</sup>

The plant *Plectranthus amboinicus* commonly known as Country borage, Indian borage is a dicotyledonous plant belonging to the family: *Lamiaceae*. It is a large succulent aromatic perennial herb, much branched, fleshy, highly aromatic pubescent herb with distinctive smelling leaves. The plant is distributed throughout India, cultivated in the gardens also. It is a folkloric medicinal plant used to treat malarial fever, hepatopathy, renal and vesical calculi, cough, chronic asthma, hiccough, bronchitis, anthelmintic, colic, convulsions, epilepsy. <sup>(3-7)</sup> The phytochemical studies reveals the presence of various flavonoids like quercetin, apigenin, luteolin, salvigenin, genkwanin and volatile oil. <sup>(8)</sup>

The available literature reveals that so far no medicinal herb has been screened for antileptospiral activity. To assess whether the plant *Plectranthus amboinicus* which has the traditional claim to treat fever like malaria, has antileptospiral activity, we performed broth micro dilution susceptibility test using *Leptospira biflexa*.

### Materials and Methods

*P. amboinicus* was collected in Andipatty canal, Theni, Tamilnadu, India. The plant was identified, authenticated by taxonomist and a voucher specimen (PCG PA 16) was deposited in the herbarium of Department of Pharmacognosy, Madurai Medical College, Madurai.

To obtain the aqueous extract 100 ml of distilled water was added to 5 grams of leaf powder of *P.amboinicus* and cold macerated for 24 hrs. The extract was filtered through whatmann No 1 filter paper and the filtrate was concentrated under reduced pressure to a brown mass and stored in the refrigerator. (28.79 %)

The volatile oil from the leaf was isolated using cocking – middleton apparatus by hydro distillation method. (0.24%)

Susceptibility Test <sup>(9)</sup>

The test was performed with 96 well round bottom microtitre plate in triplicate. It includes the,

Positive control	-	culture + Ellinghausen McCullough Johnson Harris (EMJH) medium <sup>(1)</sup>
Negative control	-	EMJH medium only.
Standard	-	culture + Amoxycillin in EMJH medium
Test 1	-	culture + Aqueous extract in EMJH medium
Test ii	-	culture + Volatile oil in EMJH medium

100µl of inoculam containing 7 days old culture of leptospires at the concentration of  $5 \times 10^8$  leptospires/ml was added to the test, std, positive control wells.

In positive control 100 µl of medium, for test 100 µl of test drug in medium, for std 100 µl of amoxycillin in medium were added. The plate was incubated at 30°C. After 3 days of incubation, 20 µl of 10 times concentrated alamar blue (S.D fine chem.) was added to all well. Alamar blue is a growth indicator dye that turns from dark blue to bright pink when viable organisms are present.

On the fifth day of incubation the color of each well was documented. MIC was recorded as the lowest concentration used that did not result in the blue to pink color change.

## Results and Conclusions

The test was performed using various concentrations by trial and error and the results suggest that no growth was present in 500 mcg/100µl of aqueous extract and 50 mcg/100µl for volatile oil of *P.amboinicus*.

The result of the current study reveals that both aqueous extract and volatile oil possess significant antileptospiral activity at 500 mcg/100µl and 50 mcg/100µl respectively. The previous researches reported in vitro antimicrobial activity against pathogenic and non pathogenic fungi and gram positive and gram negative bacteria at various dilutions.<sup>(10)</sup> It was also reported that ethanolic extract of leaves of *Plectranthus amboinicus* has exhibited good fungicidal activity against *Aspergillus flavus*, *A. terreus* and *Mucor* species.<sup>(11)</sup> Isolation and purification of the active principle responsible for the activity using bioassay guided fractionation is in progress in our laboratory. It may provide a potent drug molecule of antimicrobial especially antileptospiral drug from household herb *P.amboinicus*.

TABLE -I

Effect of various concentration of aqueous extract of *P.amboinicus* against *Leptospire*s

S.No	Concentration in mcg/100µl	Viability	Observation	Inference
1	Up to 450	+ ve	Color change (pink color)	Growth present
2.	500	- ve	No color change ( Blue color)	No growth

TABLE –II

Effect of various concentration of volatile oil of *P.amboinicus* against *Leptospire*s

S.No	Concentration in mcg/100µl	Viability	Observation	Inference
1	Up to 40	+ ve	Color change (pink color)	Growth present
2.	50	- ve	No color change ( Blue color)	No growth

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