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***In vitro* anti leptospiral activity of ethanolic extract of the leaf of *Andrographis paniculata* Nees (Acanthaceae)**

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Abstract

Leptospirosis is a zoonotic infection with a worldwide distribution that is associated with both endemic and epidemic diseases with the incidence of disease being highest in tropical climates. The MIC of ethanolic extract of the leaf of *Andrographis paniculata* Nees (Acanthaceae) for a total of 5 leptospiral serovars were tested for their susceptibility to its antileptospiral activity. Among the tested serovars *L. australis*, *L. icterohaemorrhagiae*, was found to be most susceptible to ethanolic extract and least in *L. javanica* were as moderate in *L. autumnalis*, *L. pomona*, Minimum MIC was found in *L. australis*, *L. icterohaemorrhagiae*, it was about 200µg/ml concentration. MIC was determined based on movement of leptospira. Better results were obtained only after 12hrs at lower concentration where as at 5 fold and 10 fold concentrations effect was noticed after 3 and 5 hrs respectively. Doxycycline used as standard. It was most effective and less toxic particularly for liver and kidney damaged patients.

Keywords: *Andrographis paniculata*, Ethanolic extract, anti leptospiral activity

Introduction

Leptospirosis has been recognized as an important public health problem because of its epidemic proportions and increasing incidence in both developing and developed countries. The disease was first described by Adolf Weil in 1886 when he reported an acute infectious disease with enlargement of spleen, jaundice and nephritis. Leptospira was first observed in 1907 from a post mortem of renal tissue slice. In 1908, Inada and Ito first identified it as the causative organism and in 1916 noted its presence in rats¹. The source of human leptospiral infection is infected animal urine. Leptospirosis can be transmitted by many animals such as rats, shunks, opossums, raccoons, foxes, and other vermin.² The usage of antibiotics like benzyl penicillin and doxycycline are

very effective against leptospiral members but observation of Jarish Harishmer Therefore it is necessary to search for more effective and less toxic novel antileptospiral agents that would overcome these disadvantages. The best solution for this issue is the use of herbal medicines. Medicinal plants have formed the basis of health care throughout the world since the earliest days of humanity and are still widely used with considerable importance in international trade^{3,4}. In certain African countries, however, up to 90% of the population still relies exclusively on plants as a source of medicines⁵. In India, medicinal plants are widely used by all sections of people either directly as folk remedies or in inherent indigenous systems of medicine or indirectly in the pharmaceutical preparations of modern medicines.⁶

Andrographis paniculata Nees (Acanthaceae) commonly known as Nilavembu is an annual herb. It is found in wild throughout the plains of India especially in Tamil Nadu, Karnataka, Maharashtra, Orissa and Uttar Pradesh. Various medicinal properties like antidiarrhoeal, anti-inflammatory, choleric and immunostimulant have been attributed to this plant in the traditional system of Indian Medicine. Further, reported activities are antimalarial, antihypertensive, antipyretic and antithrombotic.⁷The main objective of this present investigation is to find out the effect of ethanolic extract of the leaf of *Andrographis paniculata* Nees (Acanthaceae) for its antileptospiral activity against 5 serovars of selected *Leptospira*.

Materials and Methods

Plant Sample Collection and Identification

Plants were collected from natural habitats, Kanchipuram district, Tamil Nadu, India, and authenticated by Prof. P, Jayaraman of Plant Anatomy Research Center, Tambaram, Chennai 600 045, the voucher specimens were deposited in the same center Herbarium for further reference.

Extraction of Test Material

The fresh leaf materials were shade dried and powdered using the electric homogenizer. The powdered samples were extracted with 150 mL of solvent ethanol for 8-12 h by using the Soxhlet apparatus and the extract was kept in vacuum evaporator to get dried and which was stored in a refrigerator maintained at 4°.

Inoculum preparation:

The dark field microscopy confirmed leptospiral strains maintained in the EMJH semisolid medium was transferred to the same medium without agar (EMJH broth). The inoculated broth was examined properly after incubation at room temperature for 7 days in dark condition and the leptospiral inoculum has the final concentration of 10^6 organisms/ml.

Leptospiral serovars and Growth condition

A total of 5 leptospiral serovars were tested for their susceptibility to ethanolic extract of the leaf of *Andrographis paniculata* Nees (Acanthaceae) for its antileptospiral activity against 5 serovars of selected *Leptospira*. All the strains were obtained from National Reference Centre for leptospirosis, ICMR, Port Blair, Andaman and are maintained in this laboratory for this study and maintained by periodic sub culturing in EMJH semisolid medium.⁸

MIC determination: Susceptibility testing was used to determine the minimal amount of drugs which inhibit the maximum leptospirae (MIC). Broth microdilution testing was standardized with 96 well round bottom microtiter plates.⁸ The 5 strains under 5 different leptospiral serovars in EMJH broth of 150µl were poured in all wells (each column contain separate serovars). Each row is designated for various concentrations of ethanolic extract of the leaf of *Andrographis paniculata* Nees (Acanthaceae). In each row, 50µl of various concentrations of leaf extract (2, 4, 6, 8, 10, 15, 20, 25% of extract) were added and incubated for one hour at room temperature in dark condition. After incubation, from each well, a drop of sample was placed in the slide and observed under dark field microscope for checking the motility inhibition by comparing with the control culture serovars. The MIC of ethanolic extract of the leaf of *Andrographis paniculata* Nees (Acanthaceae) for A total of 5 leptospiral serovars were tested for their susceptibility to its antileptospiral activity against 5 serovars of selected *Leptospira* was assessed and tabulated in Table 2. Among the tested serovars *L.australis*, *L.icterohaemorrhagiae*, was found to be most susceptible to ethanolic extract and least in *L.javanica* were as moderate in *L.autumnalis*, *L.pomona*, Minimum MIC was found in *L.australis*, *L.icterohaemorrhagiae*, it was about 200µg/ml concentration. MIC was determined based on movement of leptospira. Better results were obtained only after 12 Hrs at lower concentration where as at 5 fold and 10 fold concentrations effect was noticed after 3 and 5 hrs respectively.

Figure 1: *Andrographis paniculata* Nees (Acanthaceae)



Table .1 Reference strains

S. No	Serovar	Serogroup	Strain	Genomospecies
1	Australis	Australis	Ballico	<i>L. interrogans</i>
2	Autumnalis	Autumnalis	Bankinang	<i>L. interrogans</i>
3	Pomona	Pomona	Pomona	<i>L. interrogans</i>
4	Javanica	Javanica	Poi	<i>L.borgpetersenii</i>
5	Icterohaemorrhagiae	Icterohaemorrhagiae	RGA	<i>L. interrogans</i>

Table 2: MIC of crude ethanolic extract of *Andrographis paniculata* Nees (Acanthaceae) by broth microdilution method

S. No	Serovar	Antileptospiral activity in µg/ ml
1	Australis	200
2	Autumnalis	400
3	Pomona	400
4	Javanica	600
5	Icterohaemorrhagiae	200
6	Standred(doxycycline)	400-800

Discussion

Leptospirosis is a zoonotic infection with a worldwide distribution that is associated with both endemic and epidemic diseases with the incidence of disease being highest in tropical climates. The diverse clinical presentations of this disease make it essential for the laboratory to play a role in diagnosis. Microbiological diagnosis of leptospirosis aims at demonstrating the leptospire, by culturing them or by demonstrating an appreciable antibody response to them.⁹ Laboratory diagnosis of leptospirosis is an area ill-understood by many of the workers involved in leptospirosis diagnosis and surveillance. Selection of right

specimens and tests and the correct interpretation of test results are important in order to provide better patient care. Compared to traditional broth macrodilution However, a rapid and convenient *in vitro* screening tool to assess the activity of numerous antimicrobial agents against various serovars of *Leptospira* could more expeditiously assess potential antimicrobial agents for clinical utility in humans and animals.⁸ The usage of antibiotics like benzyl penicillin and doxycycline are very effective against leptospira but reaction is high in liver and kidney damaged patients. Herbal plants and plant products¹⁰ more effective and less toxic.

Conclusion

Present study exhibit a wide rang of antileptospirosis activity. More and further studies needed to found toxic free drug from phytochemicals to cure and to protect from leptospiral infections.

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