

Cytotoxic Potential of *Andrographolide* against Bovine Tuberculosis

Ashish Shrivastava and H. K. Garg*

Research Scholar, Department of Biotechnology, CSA Govt. Post Graduate Nodal College Sehore - 466001 (India)

*Professor of Cell & Molecular Biology, Genetics and Biotechnology
Sarojini Naidu Govt. Girls Post Graduate (Autonomous) College, ShivajiNagar, Bhopal - 462016 (India)

Abstract: Bovine tuberculosis is a chronic mycobacterial infection that impinges on a wide range of mammals, including man, cattle, deer, pigs and carnivores. The present investigation deals with the anti-mycobacterial potential of *Andrographolide* against *Mycobacterium bovis*, commonly known as bovine tuberculosis. The bioactive compound *andrographolide* was isolated from the leaves of *Andrographis paniculata*. Employing different techniques viz. zone of inhibition, disc diffusion and turbidometric method, the MIC of *andrographolide* was found to be vacillating between 100 and 11.11 µg/ml against the test organism (Garg & Shrivastava, 2013). The results suggested *andrographolide* compound to be a potent antibiotic against *M. bovis* and its inhibitory effect is on par with standard drugs like isoniazid and fluoroquinolones.

Keywords: *Andrographolide*, *Andrographispaniculata*, Bovine tuberculosis, *Mycobacterium bovis*.

I. Introduction

Mycobacterium bovis is part of the *Mycobacterium tuberculosis* complex and is responsible for tuberculosis infections in several animal species, including human beings (Garnier *et. al.* 2003). Infection can occur through direct contact with an abrasion or by inhalation of contaminated air (Keller *et. al.*, 2008). However, unpasteurized dairy products have been reported to be the largest source of infectivity.

Though episodes of mycobacterial contagion are not infrequent, yet our information, with reference to relative virulence of *M. tuberculosis* and *M. bovis* in man, post-infection possibilities, immunological consequences, occurrence of man-to-man transmission and its further spread to cattle, is still inadequate (Grange, 2001). Notwithstanding the fact that plants are potential source of chemical constituents with enormous propensity to synthesize a variety of structurally diverse bioactive compounds, very little documentation has been made regarding the cure of mycobacterial tuberculosis.

Andrographispaniculata is one of the plant resources containing bioactive compound - *andrographolide* and has been used in conventional Siddha and Ayurvedic system of medicine for multiple clinical applications since medieval times (Ahmed & Aswami, 1993). The plant extract is known to exhibit anti-typhoid and anti-fungal activities. According to a study conducted at Bastyr University, *Andrographispaniculata* possesses anti-hepatotoxic, anti-biotic, anti-malarial, anti-hepatitic, anti-thrombogenic, anti-inflammatory, anti-pyretic and anti-snake venom properties (Najiv, 1999).

The present study is aimed to endorse 'andrographolide' as an effective bioactive compound and its therapeutic application in treatment of bovine tuberculosis.

II. Material And Method:

Plant extract

The leaves of *A.paniculata* were procured from nursery, dried in shade, & subsequently in hot air oven, for 30 minutes at low temperature and then pulverized into dust or powder form. The dried powder was then used for extraction with solvent ethanol (Mishra *et. al.*, 2011).

Microorganisms

The strain, *M.bovis*, used in the present study, was collected from MTCC IMTECH Chandigarh. *M.bovis* was sub-cultured and preserved in agar slants at 4°C for anti-mycobacterial study.

Chemical analysis of *Andrographolide*

Thin layer chromatography (TLC) analysis was carried out for the detection of *andrographolide*. A pre-coated plate of silica gel 60F254 (Merck) and mobile phase (chloroform : methanol : ethyl acetate - 8.0 : 1.5 : 1.0) were used. The ethanolic extracts fractionated by TLC were detected through UV radiation (Electronic UV Trans-illuminator : Rf of *andrographolide* = 0.58) (Suleet, *et.al.*, 2010).

Screening for anti-mycobacterial activity

The anti-mycobacterial activity of Andrographolide was scrutinized by using zone of inhibition, disc diffusion and turbidometric method. Isoniazid and fluoroquinolones were taken as standard drugs for valid comparisons.

In zone of inhibition method, 6×10^4 CFU of *M. bovis* was spread on Middle brook 7H11 agar media. Wells were dig on the plate with a hollow glass rod and were filled with equal concentrations of andrographolide, isoniazid & fluoroquinolones in log 2 dilution. The experiment has a separate control for optimum growth of *M. bovis*. After 21 days, radius of inhibition was measured in mm.

In disc diffusion method, sterile antibiotic discs (Himedia) were taken and filled up with 100, 33.33, 11.11, 3.77 and 1.23 µg/ml of andrographolide using micropipette. At the same time, safety measures were taken to protect the flow of the solvent extract from the outer surface of the disc. The discs were placed on the middle brook 7H11 agar plates on which the bacteria were inoculated, spread and incubated at 37°C for 21 days. The diameter of inhibition zone was measured in mm.

In turbidometric method, *M. bovis* was added at the rate of 10^8 CFU / 2ml of 7H9 broth. Equal concentration of andrographolide, isoniazid and fluoroquinolones were added in log 2 dilution. Again, the experiment has a separate control for optimal growth of *M. bovis*. After 21 days O.D. was taken at 600 nm in spectrophotometer. Inhibitory activity of andrographolide was checked on the basis of optical density.

III. Results:

Each experiment was done five times and the mean values of percentage inhibition were presented (table - 1). The results obtained from ZOI, DDA and TM were compared with standard antibiotics - isoniazid and fluoroquinolones (Garg and Shrivastava, 2013). TM was found to be more effective assay for antibacterial study of *M. bovis* than ZOI and DDM. The *Andrographolide* depicted the greatest inhibitory action (98.0 ± 0.42 %) at 100 µg/ml concentration. The positive control for isoniazid and fluoroquinolones evinced 96.47 ± 0.42 % and 93.61 ± 2.7 % inhibition respectively at the same concentration.

Table-1: Growth inhibition of *M. bovis* by *Andrographolide*, Isoniazid and Fluoroquinolones

| Concentration µg/ml | | Andrographolide Mean ± SD | Isoniazid Mean ± SD | Fluroquinolones Mean ± SD |
|---------------------|-----------------------|------------------------------|------------------------|------------------------------|
| 100 | Zone of inhibition | 87.90 ± 3.70 | 94.50 ± 2.70 | 75.20 ± 3.90 |
| | Disc diffusion method | 93.14 ± 3.10 | 95.00 ± 1.12 | 93.60 ± 2.70 |
| | Turbidometric method | 98.00 ± 0.42 | 96.47 ± 2.40 | 93.61 ± 2.70 |
| 33.33 | Zone of inhibition | 71.90 ± 4.30 | 50.80 ± 2.30 | 47.90 ± 1.20 |
| | Disc diffusion method | 83.24 ± 6.40 | 42.30 ± 1.40 | 42.30 ± 2.00 |
| | Turbidometric method | 93.80 ± 0.95 | 92.50 ± 0.30 | 42.30 ± 2.10 |
| 11.11 | Zone of inhibition | 57.30 ± 6.00 | 24.40 ± 1.15 | 46.10 ± 1.14 |
| | Disc diffusion method | 41.84 ± 0.91 | 37.10 ± 1.79 | 40.90 ± 3.20 |
| | Turbidometric method | 92.70 ± 0.40 | 90.89 ± 3.60 | 40.60 ± 3.21 |
| 3.77 | Zone of inhibition | 24.80 ± 2.40 | 16.40 ± 0.10 | 29.60 ± 5.20 |
| | Disc diffusion method | 37.12 ± 1.70 | 26.30 ± 8.00 | 33.86 ± 7.50 |
| | Turbidometric method | 82.90 ± 0.54 | 82.69 ± 0.87 | 33.90 ± 7.50 |
| 1.23 | Zone of inhibition | 17.80 ± 2.80 | 13.60 ± 0.93 | 09.40 ± 2.50 |
| | Disc diffusion method | 26.90 ± 1.90 | 21.60 ± 2.70 | 22.00 ± 1.17 |
| | Turbidometric method | 55.10 ± 5.00 | 78.39 ± 3.97 | 22.10 ± 1.14 |

Value are mean of % inhibition with ± SD (n = 5)

Figure- 1: Growth inhibition of *M. bovis* by Disc diffusion and Zone of inhibition.

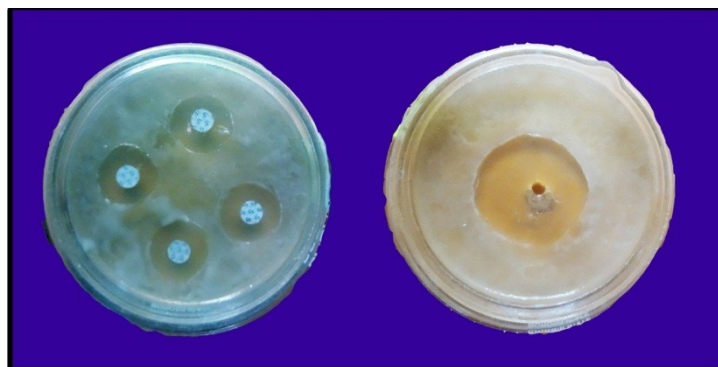


Figure- 2: Different concentration of Andrographolide, Fluoroquinolones and Isoniazid in *M. bovis* culture.

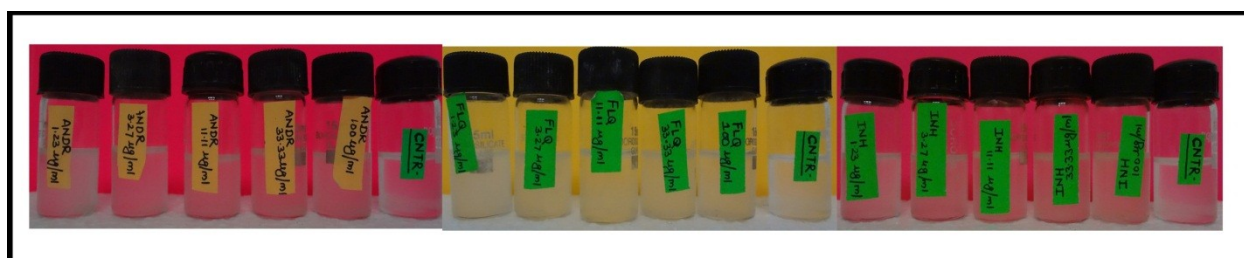
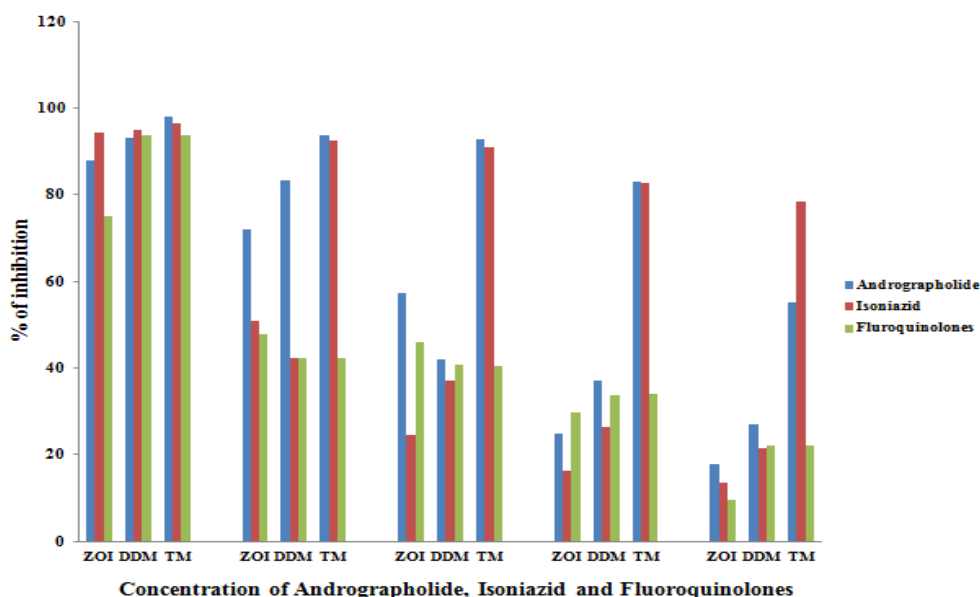


Figure- 3 : Growth inhibition of *M. bovis* by Andrographolide, Isoniazid and Fluoroquinolones.



IV. Discussion

The use of natural products as therapeutic agents has become ever more popular. However, only few plant species have been methodically investigated for their medicine properties. Gautam *et al.* (2007) reported the plant families of Asteraceae, Lamiaceae, Fabaceae and Apiaceae has anti-mycobacterial activity. Gupta *et al.* (2008) found the extracts of *A. indica*, *A. vasica*, *A. cepa*, *A. sativum* and *A. verato* exhibit anti-tuberculosis activity in L-J medium. Antony & James (2011) reported the extracts of *Alstonia scholaris* have 73.09% inhibition against clinical strain, resistant to streptomycin, isoniazid, rifampicin and pyrazinamide. The increasing incidences of MDR and XDR-TB worldwide have prompted the urgent need to look for novel anti-tuberculosis drugs. In this wake, andrographolide flares up a hope against the nuisance activities of different strains of *M. tuberculosis*.

References

- [1]. **Ahmed M. and Asmawi M.Z.(1993)**.Some pharmacological effects of aqueous of *Andrographis paniculata* Nees. International Conference on the Use of Traditional Medicines and Other Natural Product in Health Care (Abstract). University Sains Malaysia, Malaysia, 122.
- [2]. **Antony M. and James J.(2012)** .Anti-mycobacterial activity of plant *Alstoniascholaris*. Int. J. Curr. Pharm. Res., 4(1): 40-42.
- [3]. **Garg H.K. and Shrivastava A. (2013)**. Clinical use of andrographolide as a potential drug against vole tuberculosis, J. Pure Appl. Zool.1(3) 223-226.
- [4]. **Garnier T. and Eiglmeier K. (2003)**. The complete genome sequence of *Mycobacterium bovis*. Proc. Nat. Acad. Sci. USA 100:7877-7882.
- [5]. **Gautam R. and Saklani A. (2007)**. Indian medicinal plants as a source of anti-mycobacterial agents. Journal of Ethnopharmacology, 110: 200-234.
- [6]. **Grange J.M. (2001)**. *Mycobacterium bovis* infection in human beings. Pub Med. J., 81: 71-72.
- [7]. **Gupta R., Thakur B., Singh P., Singh H.B., Sharma V.D., Katoch V.M. and Chauhan S.V.S.(2008)**. Anti-tuberculosis activity of selected medicinal plants against multi-drug resistance *Mycobacterium tuberculosis* isolates. Ind. J. Med. Res., 131:809-813.
- [8]. **Keller P.M. and Bottger E.C. (2008)**. Tuberculosis vaccine strain *Mycobacterium bovis* BCG Russia is a natural rec-A mutant. BMC Microbiol., 8:120.
- [9]. **Misra C.S., Pratyush K., Sagadevan L.D.M., James J., Veettil A.K.T. and Thankamani V.(2011)**. A Comparative study on phytochemical screening and antibacterial activity of roots of *Alstoniascholaris* with the roots, leaves and stem bark, 58: 124-131
- [10]. **Najib, N.A., Rahman, N., Furuta, T., Kojima, S., Takane, K. and Ali, M.M. (1999)** Anti malarial activity of extracts of Malaysian medicinal plants J. Ethnopharmacol. 64: 249-254.
- [11]. **Sule A., Ahmed Q.U., Samah O.A. and Omar M.N. (2010)**. Screening for antibacterial activity of *Andrographis paniculata* used in Malaysian Folkloric Medicine: A possible alternative for the treatment of skin infections, Ethnobotanical Leaflets, 14 : 445-56.