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RESEARCH ARTICLE

Effect of Andrographolide on Proliferation of *Mycobacterium canetti.*

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ABSTRACT

The present investigation deals with the anti-mycobacterial prospective values of Andrographolide against *Mycobacterium canetti*, commonly regarded as smooth variant of the tuberculosis. The bioactive compound andrographolide was extracted from the leaves of *Andrographis paniculata*. Employing different techniques viz. Zone of Inhibition, Disc Diffusion and Turbidometric Method, the MIC of andrographolide varied between 100 and 11.11µg/ml against the test organism (Garg & Shrivastava, 2013). The result suggested andrographolide compound to be a potent antibiotic against *M. canetti*, as good as any standard drug like isoniazid and fluoroquniolones.

KEYWORDS

Mycobacterium canetti, Andrographolide, Andrographis paniculata, Tuberculosis

INTRODUCTION

Mycobacterium canetti, a novel pathogenic taxon of the Mycobacterium Tuberculosis Complex (MTC), was originally isolated from a 20 year old French farmer suffering from pulmonary tuberculosis by *G. canetti* in 1969. These isolates exhibited a characteristic growing pattern predominated by smooth and glossy clusters. *Per se*, MTC comprises *M. tuberculosis, M. bovis, M. caprae, M. africanum, M. microti, M. pinnipedi* and *M. canetti*. Although these species, in some way, display specific genetic homology, there are prominent phenotypic differences in their relative pathogenicity for different mammalian species.

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Andrographis paniculata is one of the plant resources containing bioactive compound andrographolide and has been used in conventional Siddha and Ayurveda for several medical applications (Ahmed & Aswami, 1993). The plant extract is known to exhibit anti-bacterial and anti-fungal properties. According to a study conducted at Bastry University, *Andrographis paniculata* possesses anti-pyretic and anti-snake venom properties too (Najib, 1999).

MATERIALS AND METHOD

Plant Extract

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

Microorganisms

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

Chemical Analysis for Andrographolide

Thin layer chromatography (TLC) analysis was carried out for the detection of andrographolide. A precoated plate of silica gel 60F254 (Merk) and mobile phase (chloroform : methanol : ethyl acetate - 8.0 : 1.5 : 1.0) were used. The ethanolic extract fractionated by TLC were detected through UV radiation (Electronic UV Tans-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 fluroquinolones were taken as standard drugs for valid comparisons.

In ZoI, 6 x 10^4 CFU of *M. canetti* was spread on middle brook 7H11 agar media. Wells were dig on the plate with a hollow glass rod and were filled with equal concentration of andrographolide, isoniazid & fluroquinolones in $\log_2$ dilution. The experiment has a separate control for optimum growth of *M. canetti*. After 21 days, radius of inhibition was measured in mm.

In DD, 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 disc 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 TM, *M. canetti* was added at the rate of 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. canetti*. After 21 days O.D was taken at 600 nm using a spectrophotometer. Inhibitory activity of andrographolide was checked on the basis of optical density.

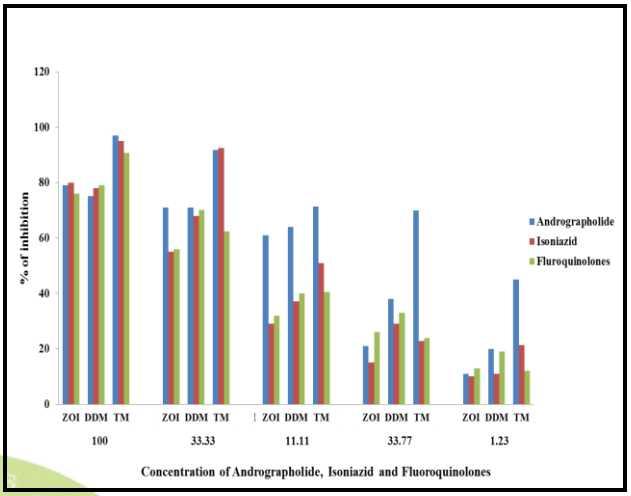

Figure-1: Growth inhibition of *M. canetti* by Andrographolide, Isoniazid and Fluoroquinolones

RESULTS

The experiments were carried out in five replicates and the mean values of percentage inhibition were presented (table-1). The results obtained from ZoI, DDM, and TM were compared with standard antibiotics-isoniazid and fluoroquinolones (Garg and Shrivastava, 2013). TM yielded more consistent results on *M. canettii* than ZoI and DDM. The *andrographolide* depicted the greatest inhibitory action (97.0 ± 0.82 %) at 100 µg/ml concentration. The positive control for isoniazid and fluoroquinolones evinced 95.47 ± 1.40 % and 90.7 ± 2.2 % inhibition respectively at the same concentration.

DISCUSSION

The use of natural products as therapeutic agents has become ever more popular. However, only few plants species have been methodically investigated for their therapeutic properties. Lall (2001) investigated anti-mycobacterial activity of diospyrin, isolated from *Eucleanatalensis* against drug-sensitive and drug-resistant strain of *M. tuberculosis*.

Table1: Growth inhibition of *M. canetti* by Andrographolide, Isoniazid and Fluoroquinolones

Concentration µg/ml		Andrographolide Mean ± SD	Isoniazid Mean ± SD	Fluroquinolones Mean ± SD
100	Zone of Inhibition	79.0 ± 1.20	80.0 ± 1.60	76.0 ± 1.20
	Disc Diffusion	75.0 ± 3.50	78.0 ± 1.60	79.0 ± 1.80
	Turbidometric Method	97.0 ± 0.82	95.4 ± 1.40	90.7± 2.20
33.33	Zone of Inhibition	71.0 ± 1.90	55.0 ± 2.30	56.0 ± 1.50
	Disc Diffusion	71.0 ± 2.20	68.0 ± 2.70	70.0 ± 5.50
	Turbidometric Method	91.7 ± 1.10	92.5 ± 0.30	62.3 ± 1.10
11.11	Zone of Inhibition	61.0 ± 2.00	29.0 ± 1.60	32.0 ± 2.40
	Disc Diffusion	64.0 ± 2.50	37.1 ± 2.50	40.0 ± 3.20
	Turbidometric Method	71.4 ± 1.40	50.9 ± 3.60	40.6 ± 3.21
3.77	Zone of Inhibition	21.0 ± 1.30	15.0 ± 2.60	26.0 ± 4.30
	Disc Diffusion	38.0 ± 4.70	29.0 ± 1.40	33.0 ± 2.50
	Turbidometric Method	69.9 ± 1.54	22.7 ± 0.47	23.9 ± 7.50
1.23	Zone of Inhibition	11.0 ± 2.20	10.0 ± 2.70	13.0 ± 1.80
	Disc Diffusion	20.0 ± 2.40	11.0 ± 2.70	19.0 ± 1.17
	Turbidometric Method	45.0 ± 1.00	21.4 ± 3.17	12.1 ± 1.14

Gautam *et. al.* (2007) studied certain plant families like *Asteraceae, Fabaceae* and *Apiaceae* for their anti-mycobacterial activity. Gupta *et. al.* (2008) found the extracts of *Allium vasica, A. cepa, A. sativum* and *A. vera* to demonstrate anti-tuberculosis activity in L-J medium. Antony & James (2011) reported the extracts of *Alstonia scholaris* to have 73.09% inhibition against clinical strain, which were resistant to streptomycin, isoniazid and pyrazinamide too. Ibrahim *et. al.* (2012) observed antibacterial activity of *S. setigera* using the Alamar Blue Assay against a virulent strain of *M. tuberculosis* (H37Rv). Recently, cytotoxic potential of andrographolide have been investigated on *M. microti* (Garg and Shrivastava, 2013) and *M. bovis* (Shrivastava and Garg, 2013). In both the studies, more than 96% inhibition was calculated at 100 µg/ml concentration.

Tuberculosis (TB) kills more or less two million people annually. Efforts to treat the disease become much more complicated due to environment of drug resistant TB strain (MDR and XDR-TB) and co-infection with HIV. There is an urgent need to search for and develop new, economical and effective anti TB drugs. In this wake, andrographolide flares up a hope against the nuisance of *M. tuberculosis*.

REFERENCES

1. Ahmad, M., & Asmawi, M. Z. (1993, June). Some pharmacological effects of aqueous extract of Andrographis paniculata Nees. In *Proceedings of the International Conference on the Use of Traditional Medicine and Other Natural Products in Health-Care. School of Pharmaceutical Science, University of Science Malaysia: Penang* (Vol. 573).

2. Antony M. and James J., (2012). Anti-mycobacterial activity of plant Alstonia scholars. International *Journal of Current Pharmacology Research*, 4(1), 40-42.

3. Garg, H. K., & Shrivastava, A. (2013). Clinical use of andrographolide as a potential drug against vole tuberculosis. *Journal of Pure & Applied Zoology*, 1(3), 223-226.

4. Garnier, T., Eiglmeier, K., Camus, J. C., Medina, N., Mansoor, H., Pryor, M., & Hewinson, R. G. (2003). The complete genome sequence of Mycobacterium bovis. *Proceedings of the National Academy of Sciences*, 100(13), 7877-7882.

5. Gautam, R., Saklani, A., & Jachak, S. M. (2007). Indian medicinal plants as a source of antimycobacterial agents. *Journal of ethnopharmacology*, 110(2), 200-234.

6. Grange, J. M. (2001). Mycobacterium bovis infection in human beings. *Tuberculosis*, 81(1), 71-77.

7. Gupta, R., Thakur, B., Singh, P., Singh, H. B., Sharma, V. D., Katoch, V. M., & Chauhan, S. V. (2010). Anti-tuberculosis activity of selected medicinal plants against multi-drug resistant Mycobacterium tuberculosis isolates. *Indian Journal of Medical Research*, 131(6). 809-813.

8. Babalola, I. T., Adelakun, E. A., Wang, Y., & Shode, F. O. (2012). Anti-TB Activity of Sterculia setigera Del., Leaves (Sterculiaceae). *Journal of Pharmacognosy and Phytochemistry*, 1(3), 19-26.

9. Keller P. M. and Bottger E. C., (2008). Tuberculosis vaccine strain Mycobaterium bovis BCG Russia is a natural rec-A mutant. *BMC Microbiol*, 8, 120.

10. Lall, N., & Meyer, J. J. M. (2001). Inhibition of drug-sensitive and drug-resistant strains of Mycobacterium tuberculosis by diospyrin, isolated from Euclea natalensis. *Journal of ethnopharmacology*, 78(2), 213-216.

11. Misra, C. S., Pratyush, K., Sagadevan, L. D. M., James, J., Veettil, A. K. T., & Thankamani, V. (2011). A comparative study on phytochemical screening and antibacterial activity of roots of Alstonia scholaris with the roots, leaves and stem bark. *International Journal of Research in Phytochemistry and pharmacology*, 1(2), 77-82.

12. Najib Nik A Rahman, N., Furuta, T., Takane, K., & Ali Mohd, M. (1999). Antimalarial activity of extracts of Malaysian medicinal plants. *Journal of ethnopharmacology*, 64(3), 249-254.

13. Shrivastava, A., & Garg, H. K. (2013). Cytotoxic Potential of Andrographolide against Bovine Tuberculosis. *IOSR Journal of Pharmacy and Biological Sciences*. 8(5), 01-04.

14. Sule, A., Ahmed, Q. U., Samah, O. A., & 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*, 8(4), 445-56.

