JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

Shukla and Sharma 2013/14 : 3 (3) 199-201

EFFECT OF SOME MEDICINAL PLANTS ON GROWTH OF MYCOBACTERIUM TUBERCULOSIS, MULTI DRUG RESISTANT MYCOBACTERIUM TUBERCULOSIS AND MYCOBACTERIUM OTHER THAN TUBERCULOSIS

*Prashant Shukla**[1], Ajay Sharma*[2]

Address(es): **Dr. Prashant Shukla**,
[1]Devleela Biotechs, VIP Road, Anand Vihar, 492001, Raipur, Chhattisgarh, India.
[2]Lovely Professional University, Phagwara, Punjab, India.

*Corresponding author: prashant19782000@gmail.com

ARTICLE INFO

Received 14. 1. 2013
Revised 5. 10. 2013
Accepted 7. 10. 2013
Published 1. 12. 2013

Regular article

OPEN ACCESS

ABSTRACT

Six plants of medicinal uses were tried for their inhibitory effect on *Mycobacterium tuberculosis* (MTB), multi drug resistant *Mycobacterium tuberculosis* (MDR MTB) and *Mycobacterium* other than tuberculosis (MOTT). MTB, MDR MTB and MOTT were cultured in 12B medium vials for Bacterc 460 TB system and incubated at 37˚C. The vials were read in Bacterc 460 TB system. Garlic, *Ocimum sanctum*, onion and neem showed effectiveness towards Mycobacterium tuberculosis and multi drug resistant Mycobacterium tuberculosis to some extent but ginger showed no effect at all. None of the plants studied had any inhibitory effect on Mycobacterium other than tuberculosis**.** *Aloe vera* had opposite effect on the growth and it was found to be assisting the growth of *Mycobacterium tuberculosis* and multi drug resistant *Mycobacterium tuberculosis*. The tests performed were in-vitro and the authors conlude that in-vivo the results may vary.

Keywords: *Mycobacterium tuberculosis*, multi drug resistant *Mycobacterium tuberculosis*, *Mycobacterium* other than tuberculosis

INTRODUCTION

Tuberculosis is a major health problem which results in millions of death per year. Ninety five percent of cases are in third world countries where due to inadequate health care facilities and contact tracing drug resistant tuberculosis is increasing (**Nouvel *et al.*, 2006**). With more than one third of human population infected with tubercle bacillus and HIV infection intensifying the problem need of plant based antimycobacterials is increasing. This need is also highlighted due to emergence of multi drug resistant (MDR), extreme drug resistant (XDR) and recently reported Total drug resistant (TDR) tubercle bacillus in different parts of the world (**Pandya *et al.*, 2012**). Various workers have been working on this very problem and different plant based antimycobacterials have been discovered (**Rivero-cruz *et al.*, 2003; Graham *et al.*, 2003**) Garlic (**Hannan *et al.*, 2011**); (**Dhamija *et al.*, 2010; Gupta et al., 2010**), ginger (**Hiserodt *et al.*, 1998**), Ocimum (**Prakash and Gupta, 2005**), onion (**Gupta *et al.*, 2010**).
The objective of our study was to determine the effect of extracts of some medicinal plants on *Mycobacterium tuberculosis* and multi drug resistant *Mycobacterium tuberculosis*.

MATERIAL AND METHODS

Mycobacterium tuberculosis (MTB), multi drug resistant *Mycobacterium tuberculosis* (MDR MTB) and *Mycobacterium* other than tuberculosis (MOTT) used in the current study were isolated from the clinical samples of the patients sent to the laboratory for diagnosis using 12B (Mycobacterial Middlebrook 7H 12 medium) medium vials for Bactec 460 (Becton, Dickinson & Co., Towson, MD). The samples were recultured by inoculating 100µl of stored pathogenic culture into fresh 12B medium vials prior to the study. *Azadirachta indica* (Neem, leaves and thin green stems) *Ocimum sanctum* (leaves only), *Aloe vera*, (gel from plant), *Allium cepa* (Onion, root) *Zingiber officinale* (Ginger, root) *Allium sativum* (Garlic, clove) were used in the study to determine their anitmycobacterial property against MTB, MDR MTB and MOTT. Ethanol (70%) and double distill water were used for isolating the plant extracts.

Plant extracts

Six different plant were used in the current study as described above. Each plant material was chopped into small pieces, air dried. Then the samples were grounded with either ethanol or double distill water to make the stock solution of 1 g plant/ 10ml of solvent. The plant extracts were stored at 4˚C till further use. Control was set up each set of experiments with distill water and ethanol added in place of the extracts.

Mycobacterium cultures

Mycobacterium cultures used in the current study were previously isolated from the clinical samples sent to the laboratory for diagnosis. The samples were inoculated in 12B medium vials and incubated at 37˚C. The growth of microorganisms in the vials were tested through Bacterc 460 TB system (Becton, Dickinson & Co., Towson, MD) every week. The positive cultures were confirmed by Ziehl-Neelsen staining method for the presence of *M. tuberculosis* (**Ellis and Zabrowarny, 1993**). The positive cultures were tested for sensitivity againsts 13 different first and second line drugs to confirm MTB and MDR MTB (Table 1). NAP (p-nitro-a-acetylamino-B-hydroxy-propiophenone) test was used to confirm MOTT (**Stager *et al.*, 1988**). The previously kep cultures were then subcultured in 12B medium vials and incubated at 37˚C.
The 100µl of the inoculums from the subculture and a fixed volume of plant extract (0.25, 0.50 and 0.75 µl) were inoculated into fresh 12B media vials. Control was kept for the three species where the species were inoculated in fresh vials not containing any extracts.

Table 1 Drugs used in study

Type	Drugs
First Line	Streptomycin
	Rifampicin
	Pyrazinamide
	Isoniazid
	Ethambutol
Second Line	Ofloxacin
	Capreomycin
	Pyrazinamide
	Cycloserine
	ρ-aminosalicylic acid (PAS)
	Leofloxacin
	Ethionamide
	Kanamycin
	Moxifloxacin

Calculations

Formula used for calculating percentage inhibition:-(Difference in Growth Index (GI) of control and sample/ Growth Index (GI) of control)*100. (Data not shown)

RESULTS AND DISCUSSION

The readings of BACTEC 460 TB system was used for the final result preparation. Any GI reading above 200 was considered positive growth.

Effect of extracts on MTB

There was significant reduction in growth rate in the case of samples in which alcoholic extracts of tulsi (Ocimum sanctum), garlic and neem were added at 0.25 µl (Table 2) and also with aqueous extract of garlic growth was evident after 3rd week. With rest of the samples containing 0.25 µl extracts maximum growth was found within 2nd week of inoculation. Samples containing 0.50 µl of alcoholic neem, Ocimum sanctum, garlic and onion showed very much reduced growth along with the aqueous extract of garlic. With the samples containg 0.75 µl of extracts with the exception of aloe vera and ginger all had inhibitory effect on Mycobacterium tuberculosis (Table 3).
Only garlic was able to inhibit MDR growth at 0.25 µl extract. Ocimum sanctum had some inhibitory effect but the bacterium was able to grow after 4 weeks of incubation. With 0.50 and 0.75 µl extracts inhibition of MDR MTB was evident in alcoholic extracts of neem, garlic and onion and aqueous extract of Ocimum sanctum with readings lower in the case of samples containing 0.75 µl of extracts (Table 4, 5). Results of controls and MOTT are not shon as they were positive for growth for eavery sample.

Table 2 Percent reduction in growth rate of MTB due to effect of aqueous extracts of different plants at0.25, 0.50 and 0.75 microgram of extract per ml of media

Sample	Extract microgram of media	**Percentage reduction of growth in MDR TB**
Neem	0.25	No reduction
	0.50	No reduction
	0.75	82
Tulsi	0.25	No reduction
	0.50	No reduction
	0.75	87
Garlic	0.25	88
	0.50	91
	0.75	100
Ginger	0.25	No reduction
	0.50	No reduction
	0.75	30
Onion	0.25	85
	0.50	89
	0.75	100
Aloe vera	0.25	100% Increase in growth
	0.50	100% Increase in growth
	0.75	100% Increase in growth

Legend: MTB –*Mycobacterium tuberculosis*

Table 3 Percent reduction in growth rate of MTB due to effect of alcoholic extracts of different plants at 0.25, 0.50 and 0.75 microgram of extract per ml of media

Sample	Extract microgram of media	**Percentage reduction of growth in MDR TB**
Neem	0.25	72
	0.50	85
	0.75	93
Tulsi	0.25	81
	0.50	84
	0.75	90
Garlic	0.25	96
	0.50	96
	0.75	97
Ginger	0.25	No reduction
	0.50	No reduction
	0.75	27
Onion	0.25	90
	0.50	92
	0.75	93
Aloe vera	0.25	100% Increase in growth
	0.50	100% Increase in growth
	0.75	100% Increase in growth

Legend: MTB –*Mycobacterium tuberculosis*

Table 4 Percent reduction in growth rate of MDR TB due to effect of aqueous extracts of different plants at 0.25, 0.50 and 0.75 microgram of extract per ml of media

Sample	Extract microgram of media	**Percentage reduction of growth in MDR TB**
Neem	0.25	No reduction
	0.50	No reduction
	0.75	6
Tulsi	0.25	58
	0.50	67
	0.75	90
Garlic	0.25	90
	0.50	94
	0.75	98
Ginger	0.25	No reduction
	0.50	No reduction
	0.75	No reduction
Onion	0.25	63
	0.50	79
	0.75	80
Aloe vera	0.25	100% Increase in growth
	0.50	100% Increase in growth
	0.75	100% Increase in growth

Legend: MDR TB – Multi drug resistant *Mycobacterium tuberculosis*

Table 5 Percent reduction in growth rate of MDR TB due to effect of alcoholic extracts of different plants at 0.25, 0.50 and 0.75 microgram of extract per ml of media

Sample	Extract microgram of media	**Percentage reduction of growth in MDR TB**
Neem	0.25	78
	0.50	82
	0.75	84
Tulsi	0.25	No reduction
	0.50	No reduction
	0.75	No reduction
Garlic	0.25	83
	0.50	90
	0.75	95
Ginger	0.25	No reduction
	0.50	No reduction
	0.75	No reduction
Onion	0.25	77
	0.50	84
	0.75	87
Aloe vera	0.25	100% Increase in growth
	0.50	100% Increase in growth
	0.75	100% Increase in growth

Legend: MDR TB – Multi drug resistant *Mycobacterium tuberculosis*

CONCLUSION

It is evident from the study that the plant extracts used had some inhibitory effect of MTB as well as MDR MTB but had no inhibitory effect on MOTT species. The extracts of *Ocimum sanctum*, garlic, neem and onion inhibited the growth of MTB at different concentrations of the extracts as was the case even with MDR MTB. At 0.75 µl concentration aqueous extract showed inhibitory effect on MDR MTB while at the same concentration alcoholic extract of Ocimum sanctum did not produce inhibitory effect on the same organism. Therefore it can be concluded that the inhibitory molecule of MDR MTB is water soluble and it is not present in the alcoholic extract. The results show that further work if done with these plants, some molecules could be isolated which can prove to be effective against MTB as well as MDR MTB.

REFERENCES

ARUNKUMAR, S., MUTHUSELVUM M. 2009. Analysis of Phytochemical Constituents and Antimicrobial Activities of *Aloe vera* L. Against Clinical Pathogens. *World J. of Agri. Sci.* 5(5), 572-576.
DHAMIJA, P., SETHI, S., MEHARWAL, S., KUMAR, S., MALHOTRA, S., SHARMA, M., PANDHI, P. 2010. Crude aqueous extract of garlic reduces MIC

of isoniazid and rifampicin against *Mycobacterium tuberculosis* by broth microdilution method. *J Compl Integr Med*, 7(1), Article 47.

ELLIS, R. C., ZABROWARNY, L. A. 1993 Safer staining method for acid Fast bacilli. *J.Clin Pathol*, 46,559-560.

GRAHAM, J.G., PENDLAND, S. L., PRAUSE, J.L., DANZINGER, L. H., SCHUNKE VIGO, J., CABLESES, F., FARNSWORTH, N. R. 2003. Antimycobacterial evaluation of Peruvian plants. *Phytomedicine*, 10(6-7), 528-535.

GUPTA, R., THAKUR, B., SINGH, P., SINGH, H.B., SHARMA, V.D., KATOCH, V.M., CHAUHAN, S.V.S. 2010. Anti-tuberculosis activity of selected medicinal plants against multi-drug resistant *Mycobacterium tuberculosis* isolates. *Ind. J. Med. Res,*. 131, 809-813.

HANNAN, A., IKRAM ULLAH, M., USMAN, M., HUSSAIN, S., ABSAR, M., JAVED, K. 2011. Anti-mycobacterial activity of garlic (*Allium sativum*) against multi-drug resistant and non-multi-drug resistant *Mycobacterium tuberculosis*. *Pak. J. Pharm. Sci*., 24(1), 81-85.

HISERODT, R. D., FRANZBLAU, S. G., ROSEN, R. T. 1998. Isolation of 6-, 8-, and 10-gingerol from ginger rhizome by HPLC and preliminary evaluation of inhibition of Mycobacterium avium and Mycobacterium tuberculosis. *J. Agric. Food Chem*., 46, 2504-2508.

NOUVEL, L.X., , KASSA-KELEMBHO, E., VULTOS, T.D., ZANDANGA, G., RAUZIER, J., LAFOZ, C., MARTIN C., BLAZQUEZ, J., TALARMIN, A., GICQUEL B. 2006. Multidrug-resistant Mycobacterium tuberculosis, Bangui, Central African Republic. *Emerging Infectious Diseases*, 12(9), 1454-1456.

PANDYA, K., PATEL, P., PATEL, G.,PARIKH, V. 2012 *In vitro* anti-mycobacterial study of essential oil of few selcted plants. *IJUPLS*, 2(2), 523-529.

PRAKASH, P., GUPTA, N. 2005 Therapeutic uses of *Ocimum sanctum* Linn (tulsi) with a note on eugenol and its pharmacological actions: A short review. *Indian J. Physiol Pharmacol*, 49(2), 125-131.

RIVERO-CRUZ, I., ACEVEDO, L., GUERRERO, J. A., MARTINEZ, S., PEREDA MIRANDA, R., MATA, R., BYE, R., FRANZBLAU, S., TIMMERMANN. 2005 Antimycobacterial agents from selected Mexican medicinal plants. *J. Pharma. Pharmacol*., 57(9), 1117-1126.

STAGER, C.E., DAVIS, J. R., SIDDIQI, S. H. 1988 Identification of Mycobacterium tuberculosis in sputum by direct inoculation of the BACTEC NAP vial. *Diagn Microbiol Infect Dis*.,10(2), 67-73.