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Anti-tuberculosis activities of the crude methanolic extract and purified fractions of the bulb of *Crinum jagus*

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Summary: Tuberculosis (TB) is of great public health burden globally especially in developing countries of Africa and Asia . Current TB regimen involves multiple therapies and of long duration leading to poor patient adherence. There is also the challenge of multidrug resistant TB. Hence, there is a need for discovery of new anti- TB drugs. This study was designed to investigate the in –vitro activity of the crude methanolic extract and chromatographic fractions of the bulb of *Crinum jagus* against *Mycobacterium tuberculosis* isolates. The extracts were screened for anti- TB activity against three different *M. tuberculosis* isolates and a drug susceptible reference strain H37Rv using Lowenstein Jensen (L-J) medium and Middlebrook 7H10agar. The crude extract was prepared using soxhlet extraction apparatus while the purified fractions were obtained by column chromatography. The two media were inoculated with *M. tuberculosis* strains, after which the crude and purified extracts were added. After 4-6 weeks incubation, colony forming units were counted and percentage inhibition calculated. The crude extract and the purified fractions showed inhibitory activity on all the isolates tested including the reference strain. Fraction 3 showed the highest inhibitory percentage (86%) among the extracts. At a concentration of 1.0mg/ml, the percentage inhibition of fraction 3, rifampicin and isoniazid against *M. tuberculosis* strain 3 were 83%, 95% and 86% in L-J medium respectively while 86%, 96% and 89% were obtained respectively in Middle brook medium. Results showed that the crude methanolic extract and the purified fractions of the bulb of *Crinum jagus* exhibited anti-mycobacterial activity which is an indication of promising potential of this plant for the development of anti-tuberculosis agent.

Keywords*: Crinum jagus*, Chromatographic fractions, *Mycobacterium tuberculosis, In-vitro*

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INTRODUCTION

Tuberculosis (TB) is a common and deadly infectious disease caused by various strains of *Mycobacterium tuberculosis* in humans (Kumar et al, 2007). It is a global health problem killing about 3 million people per year worldwide.Trends in the incidence of TB together with the development of multi drug resistant (MDR) and extensively drug resistant (XDR) strains of *M. tuberculosis* and HIV co- infection raises the need to intensify the search for more efficient drugs to combat this disease (Corbett et al, 2003; CDC, 2005; Furin, 2007).

There are claims by traditional healers in Africa that TB can be treated using herbs however, these claims have no scientific justification (Faleyimi et al, 2009; John et al, 2010). This study was prompted by an ethno-botanical survey that reported *Crinum jagus* is one of the plants used traditionally for the treatment of TB in Southern parts of Nigeria (Idu et al, 2010).

Crinum is a genus of about 180 species of perennial plants that have large- showy flowers on leafless stem and develop from bulbs. The genus Crinum belongs to the family Amaryllidaceae, Phylum Angiospermae and Sub-phylum Lilifloral. Several species are cultivated as ornamentals and for medicinal purposes (Burkill, 1985; Ghosal et al, 1985; Tram et al, 2002 and Fennel and Staden, 2001). *Crinum jagus* are bulbous plants with umbels of lily-like flowers, its' local name is *Ogede Odo* in Yoruba. They are found in tropical and subtropical regions of the world where for centuries, they have been used to cure certain ailments and diseases such as asthma and sickle cell disease (Adesanya et al, 1992).

The plant attracts attention due to various medicinal properties such as antibacterial, antifungal (Adesanya et al, 1992), anticholinergic (Peter et al, 2004), anticonvulsant (Edema and Okiemen, 2002), anti- snake venom (Ode and Asuzu, 2006), anti - asthmatic (Ogunkunle and Olopade, 2011) and

antioxidant activities (Ode and Asuzu, 2010).In this study the anti-TB activity of the crude methanolic extract and purified fractions of the bulb of *Crinum jagus* was tested against three *M. tuberculosis* isolates.

MATERIALS AND METHODS

Plant Material

The bulb of *Crinum jagus* were collected from Omi Adio, a town located at the suburb area in Ibadan, Oyo state, Nigeria and were authenticated at the Forestry Research Institute of Nigeria (FRIN), Ibadan. Voucher specimen of the plant (FHI -10911) was deposited in the herbarium of FRIN, Ibadan, Oyo state.

Growth Media, Mycobacterial isolates and Drugs

Commercial powder of both Lowenstein Jensen (L-J) medium and Middle brook 7H10 agar used for the antimicrobial assay were obtained from Sigma Chemicals, USA. They were prepared according to manufacturer's instructions.

The three *M. tuberculosis* strains and the reference drug susceptible strain H37Rv used for the study were obtained from TB Reference Laboratory (South-West Zone), Medical Microbiology Department, University College Hospital, Ibadan. Nigeria. The standard anti-TB drugs used for the study, rifampicin and isoniazid were obtained from Themedica Pharmaceutical Drugs, India.

Preparation of crude extract

Fresh samples of the plant materials were chopped. The chopped samples were air-dried and ground into powdery forms. The powdered plant was loaded into soxhlet extractor and then extracted with boiling petroleum ether, followed by methanol. The free methanol was allowed to evaporate using water- bath, a brown viscous solid was obtained, it was transferred into a clean dry bottle, weighed and labelled as crude methanolic extract.

Preparation of purified fractions

The crude methanolic extract of the plant was purified by column chromatography and thin layer chromatography (TLC). Glass column was packed with silical gel which then adsorbed the crude extract. It was then packed onto the column layer. The mobile phase consisted of three solvents, hexane, ethyl-acetate and methanol mixed in different proportions. The various proportions of the solvents were pushed through the bed. Twenty one fractions were obtained. The fractions were pooled together by thin layer chromatography (TLC). This reduced the number of the fractions to five. Fractions 3, 4 and 5 were used to test for biological activities.

Preparation of drugs/ extracts

Crude extract (10mg) and each of the chromatographic fractions (fractions 3, 4 and 5) were separately dissolved in 10ml of methanol to obtain a stock solution of 1.0mg/ml. From the stock solution, various concentrations (0.2mg/ml, 0.4mg/ml, 0.6mg/ml, 0.8mg/ml and 1.0mg/ml) of the crude extract and each of the fraction was obtained. Stock solutions of rifampicin and isoniazid (1.0mg/ml) were separately prepared from which various concentrations (0.2 mg/ml, 0.4 mg/ml, 0.6 mg/ml, 0.8 mg/ml, and 1.0 mg/ml) were obtained and used for the susceptibility tests.

Preparation of Culture Media

The culture media used for anti-TB assay - Lowenstein Jensen (L-J) medium and Middle book 7H10 agar were prepared according to the manufacturer's instruction (Sigma Chemicals, USA).

Phytochemical Test

The extract was screened for the presence of alkaloids, flavonoids, saponins, phenols, tannins, steroids, protein, carbohydrates, cardiac glycosides as described by Edeoga et al, (2005).

Antimicrobial Assay

Evaluation of anti-tuberculosis activities of the crude extract and purified fractions in Lowenstein Jensen (L-J) medium.

Tenfold dilutions of standard l.0mg/ml of each strain of *Mycobacterium tuberculosis* suspensions including H37Rv reference drug susceptible control strain were prepared as described by Canetti et al (1969). One loopful (60µl) of this suspension was streaked on the L-J slants using 3mm external diameter loop. The crude extract, chromatographic fractions (fractions 3, 4 and 5) and the standard drugs at various concentrations of 0.2mg/ml, 0.4mg/ml, 0.6mg/ml,0.8mg/ml and 1.0mg/ml were separately incorporated in the medium. The media were incubated at 37^0C for 42 days. For comparison extract free slants were used as controls. Each week, the cultures were examined for possible growth of *Mycobacteria tuberculosis* and the number of bacteria colony in each slant were counted and recorded. Percentage inhibition was calculated by mean reduction in number of colonies on extract containing medium as compared to extract free controls (Gupta et al, 2010).

Evaluation of anti-tuberculosis activities of the crude extract and purified fractions on Middle brook 7H10 Agar.

Tenfold dilutions of standard l.0mg/ml *M. tuberculosis* suspensions were prepared, using the disc diffusion method as described by Claude et al, 2012. Briefly, discs were separately impregnated with 20µl of rifampicin and isoniazid. The extract and the fractions were then left to dry for 24 hours. The Middlebrook 7H10 agar was poured onto petri dish and then divided into quadrants. The solidified medium in the quadrant was inoculated using a swab.

A rifampicin impregnated disc was placed in the first quadrant, isoniazid in the second quadrant, the third quadrant has the extract/ fractions impregnated disc while the fourth quadrant contained a blank disc that served as negative control. This was done for varying concentrations of the extract, the fractions and the standard drugs. The petri dishes were sealed with a carbon-dioxide permeable tape, left overnight in biosafety hood to allow diffusion of the extract, fractions. It was then incubated at 37^0C in a carbon-dioxide incubator for 4 weeks. The susceptibility of *M. tuberculosis* isolates to the extract and the drugs was determined by counting the number of bacteria in each quadrant using a method described by Claude et al, (2012). Percentage inhibition was calculated by mean reduction in number of colonies on extract containing as compared to extract free controls.

RESULTS

Using LJ medium, anti-TB activity of the crude extract showed that *M. tuberculosis* strain 3 had the highest inhibition of 57% representing 58 colony forming units (cfu) at a concentration of 1.0mg/ml while 95% and 84% inhibition rates which correspond to 6 and 24 cfu were recorded for rifampicin and isonazid at the same concentration respectively (Table 1).

Table1: Anti –tuberculosis activity of the crude methanolic extract of the bulb of *Crinum jagus* in Lowenstein Jensen (L-J) medium

Extract/ drug	**Isolates**	**Mean cfu on media**						**Percentage inhibition (%)**				
			Treatment concentration (mg/ml)					**Treatment concentration (mg/ml)**				
		Control	**0.2**	**0.4**	**0.6**	**0.8**	**1.0**	**0.2**	**0.4**	**0.6**	**0.8**	**1.0**
Crude Extract	H37Rv	125	110	90	82	73	54	12	28	34	42	57
	MTB1	122	108	95	80	66	56	13	22	34	45	54
	MTB2	138	121	104	88	72	61	12	25	36	48	56
	MTB3	136	120	107	84	70	58	12	25	38	49	57
Rifampicin	H37Rv	140	62	50	36	20	12	56	64	74	86	91
	MTB1	140	86	54	45	30	20	39	61	68	79	86
	MTB2	134	75	50	38	22	14	44	63	72	84	88
	MTB3	130	62	42	20	12	6	52	68	85	91	95
Isonazid	H37Rv	140	72	60	46	30	20	49	57	67	79	86
	MTB1	132	112	84	75	54	40	15	36	43	59	70
	MTB2	150	84	65	52	48	36	44	57	65	68	77
	MTB3	146	70	56	40	35	24	52	62	73	76	84

MTB = *Mycobacterium tuberculosis*, cfu = colony forming unit , % inhibition= $\frac{Cc-Ct}{Cc} X100$ Cc = No of colony in the control medium, Ct = No of colony in test medium

Table2: Anti-tuberculosis activity of the purified fractions of the bulb of *Crinum jagus* in Lowenstein Jensen (L.J) medium

Treatment	**Isolates**	**Mean cfu on media**						**Percentage inhibition (%)**				
			Treatment Concentration (mg/ml)					**Treatment concentration (mg/ml)**				
		Control	**0.2**	**0.4**	**0.6**	**0.8**	**1.0**	**0.2**	**0.4**	**0.6**	**0.8**	**1.0**
Fraction 3	H37Rv	130	70	62	46	30	22	46	52	65	77	83
	MTB1	164	102	96	86	69	45	38	41	48	58	73
	MTB2	118	75	58	45	40	25	36	51	62	66	79
	MTB3	162	82	70	58	40	25	49	57	64	75	83
Fraction 4	H37Rv	128	80	74	55	41	30	38	42	57	68	77
	MTB1	128	108	102	78	76	64	16	20	23	41	50
	MTB2	120	101	88	80	65	54	16	18	33	46	55
	MTB3	138	116	110	89	73	58	16	20	35	47	58
Fraction 5	H37Rv	120	95	83	74	50	39	21	31	38	58	68
	MTB1	120	90	74	64	52	42	25	38	47	57	65
	MTB2	122	92	68	63	45	42	25	44	48	63	66
	MTB3	142	98	76	67	52	44	31	46	53	63	69
Rifampicin	H37Rv	140	62	50	30	20	12	56	64	74	86	91
	MTB1	140	86	54	45	30	20	39	61	68	79	86
	MTB2	134	75	50	38	22	14	44	63	72	84	88
	MTB3	130	62	42	20	12	6	52	68	85	91	95
Isoniazid	H37Rv	140	72	60	46	30	20	49	57	57	79	86
	MTB1	132	112	84	75	54	40	15	36	65	68	70
	MTB2	150	84	65	52	48	36	44	57	65	68	77
	MTB3	146	70	56	40	35	24	52	62	73	76	84

MTB = *Mycobacterium tuberculosis*, cfu = colony forming unit , % inhibition= $\frac{Cc-Ct}{Cc} X100$ Cc = No of colony in the control medium, Ct = No of colony in test medium

Table 3: Anti- tuberculosis activity of the crude methanolic extract of the bulb of *Crinum jagus* in Middle brook 7H10 agar medium

Treatment	Isolates	Mean cfu on media						Percentage inhibition (%)				
			Treatment concentration(mg/ml)					Treatment concentration (mg/ml)				
		Control	0.2	0.4	0.6	0.8	1.0	0.2	0.4	0.6	0.8	1.0
Crude Extract	H37Rv	124	101	92	78	60	55	19	26	37	52	56
	MTB1	120	108	92	78	64	54	10	23	35	47	56
	MTB2	141	122	102	84	70	59	14	27	40	50	58
	MTB3	138	116	98	82	68	56	16	28	41	51	59
Rifampicin	H37Rv	126	58	40	31	15	15	54	68	75	88	96
	MTB1	130	74	52	40	29	14	43	60	69	78	89
	MTB2	140	65	50	39	25	12	54	64	72	82	91
	MTB3	132	55	40	29	20	04	58	70	78	85	97
Isonazid	H37Rv	136	68	55	40	25	15	50	60	71	82	89
	MTB1	138	105	80	62	51	38	24	42	55	63	73
	MTB2	146	80	71	60	49	31	45	52	59	66	79
	MTB3	144	68	51	39	22	16	53	65	73	85	89

MTB = *Mycobacterium tuberculosis*, cfu = colony forming unit , % inhibition= $\frac{Cc-Ct}{Cc} X100$ Cc = No of colony in the control medium, Ct = No of colony in test medium

Table 4: Anti-tuberculosis activity of the purified fraction of the bulb of *Crinum jagus* in Middle brook 7H10 agar medium

Treatment	Isolates	Mean cfu on media						Percentage inhibition (%)				
			Treatment concentration (mg/ml)					Treatment concentration (mg/ml)				
		Control	0.2	0.4	0.6	0.8	1.0	0.2	0.4	0.6	0.8	1.0
Fraction 3	H37Rv	128	65	42	31	22	12	49	67	76	83	91
	MTB1	158	104	94	82	65	37	34	41	48	59	76
	MTB2	122	69	60	44	39	24	43	51	64	68	80
	MTB3	154	78	68	52	38	22	49	59	66	75	86
Fraction 4	H37Rv	140	94	76	60	50	30	32	46	57	64	79
	MTB1	131	112	98	90	72	58	15	25	31	45	56
	MTB2	122	95	86	72	63	50	22	29	41	48	59
	MTB3	136	102	95	77	68	50	25	30	43	50	63
Fraction 5	H37Rv	138	109	92	75	61	40	21	33	46	56	71
	MTB1	138	94	78	62	58	45	25	38	51	62	68
	MTB2	146	80	71	60	49	31	45	52	59	66	79
	MTB3	144	68	51	39	22	16	53	65	73	85	89
Rifampicin	H37Rv	140	62	50	30	20	12	56	64	74	86	91
	MTB1	140	86	54	45	30	20	39	61	68	79	86
	MTB2	134	75	50	38	22	14	44	63	72	84	88
	MTB3	130	62	42	20	12	6	52	68	85	91	95
Isoniazid	H37Rv	140	72	60	46	30	20	49	57	57	79	86
	MTB1	132	112	84	75	54	40	15	36	65	68	70
	MTB2	150	84	65	52	48	36	44	57	65	68	77
	MTB3	146	70	56	40	35	24	52	62	73	76	84

MTB = *Mycobacterium tuberculosis*, cfu = colony forming unit , % inhibition= $\frac{Cc-Ct}{Cc} X100$ Cc = No of colony in the control medium, Ct = No of colony in test medium

For the anti-TB activity of the purified fractions against the tested mycobacterial isolates; *M. tuberculosis* strain 1 had the highest mean cfu of 45 on LJ medium with 73% inhibition for purified fraction 3 at 1.0mg/ml concentration while 83% inhibition and 25 mean cfu were recorded for *M. tuberculosis* strain 3 using the same fraction. This result compared favorably with 83.0% inhibition and 22 cfu obtained for control strain H37Rv. Furthermore, 6 mean cfu on LJ medium incorporated with rifampicin was recorded for *M. tuberculosis* strain 3 with 95% inhibition rate while the same isolate had a mean 24 cfu and 84% inhibition in isoniazid- embedded agar (Table2).

For the anti-TB activity of the crude extract on Middlebrook medium, *M. tuberculosis* strain 3 had highest inhibition rates of 59%, 97% and 89% at concentration of 1.0mg/ml for crude extract, rifampicin and isoniazid with 56, 4 and 16 cfu respectively (Table 3). Table 4 showed various anti-TB activities of the purified fractions in Middlebrook 7H10 medium against mycobacterial isolates tested.

DISCUSSION

Tuberculosis (TB) is a serious public health problem with medical, sociological and economic

consequences. Plants have long been valuable sources for novel medicines. Many people in developing world rely on the use of traditional medicines for treatment of infectious diseases. We present report of a study on anti-tuberculosis activity of extract of *Crinum jagus*.

In this study, we found the highest inhibition rate of 57% on LJ medium at 1.0mg/ml concentration for *M. tuberculosis* strain 3 (Table 1) while similar figure was obtained using Middlebrook agar (Table 3). Even though, the inhibition rate of 57% obtained for crude extract was lower than that of the two anti-TB drugs tested (rifampicin and isoniazid) (Table 1), the fact remains that the crude extract possesses some anti-TB properties. The lower anti-TB inhibition rate exhibited by the crude extract may be due to presence of impurities which may reduce the potency of the extract. That activity of purified fraction 3 showed 83% inhibition against *M. tuberculosis* strain 3 which was similar to 95% and 84% obtained for rifampicin and isoniazid respectively (Table 3) further reinforces its' potential anti- TB potency.

Rifampicin and isoniazid are two major anti-TB drugs. However, resistance to these two drugs have lead to development of multi-drug resistant *M. tuberculosis* (MDR-TB). MDR-TB is of great public health importance worldwide most especially in high burden countries with inadequate implementation of Directly Observed Treatment Short Course strategy (DOTS), which is the World Health Organization's strategy for global management of TB.

The emergence of MDR-TB further reinforces the need for search for new anti-TB medicines. Moreso the advent of HIV infection have increased the incident of TB. The current anti-TB drugs do not address the tenacious problems in the management of TB/HIV co-infections, because the current anti-TB drugs were manufactured years before the advent of HIV infection (Zhenkum, 2010). Hence, there is a strong need for new anti-TB drugs that would address long duration of therapy, co-administration with anti retroviral drugs in TB/HIV co-infections and to also eliminate resistant strains.

The crude methanolic extract and the purified fractions of *Crinum jagus* demonstrated a dose-dependent inhibition of the *Mycobacterium tuberculosis* isolates at various concentrations in both Lowensen Jensen (L-J) and Middlebrook 7H10 media. The anti-TB activity of the plant may be due to bioactive components such as alkaloids, flavonoids, saponins, and phenols. Further studies are needed to identify the active anti-TB agents present in the bulb of the plant.

In conclusion, results of this study showed that both the crude and purified fractions of the bulb of the plant *Crinium jagus* have anti-TB activities which may be explored for development of new anti-TB drugs.

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REFERENCES

Adesanya S.A., Olugbode T.A., Odebiyi O.O., and Aladesanmi, J.A. (1992). Antibacterial alkaloids in *Crinum jagus*. *International J. Pharmacognosy* 4: 303-307.

Burkill H.M. (1985). The useful plants in West Tropical Africa. In: Burkill HM; ed. Royal Botanic Gardens. 2nd edition, Great Britain. 63-67

Canetti G., Fox W., Khomenko A., Mahler H. T., Menon N.K., Mitchison D.A. (1993). Advances in techniques of testing mycobacterial drug sensitivity and the use of sensitivity tests in tuberculosis control programmes. *Bull. World Health Organ*. 41

Centre for Disease Control and Prevention (CDC) (2005).Worldwide emergence of *Mycobacterium tuberculosis* with extensive resistance to second line drugs. Morbidity and Mortality Weekly Report 55: 250-263.

Kirimuhuzya C., Bunalema L., Waako P., Tabuti J.R.S., Orodho J., Magadula J.J., Otieno J.N., Okemo P. (2012). Efficacy of *Cryptolepis sanguinolenta* root extract on slow-growing rifampicin resistant *Mycobacterium tuberculosis. Journal of Medicinal Plant Research*, 6(7), 1140-1146.

Corbett E.L., Watt C.J., Walker N., Maher D., Williams B.G., Raviglione M.C., Dye D. (2003). The growing burden of tuberculosis. Global trends in interactions with HIV. *Arch. Inter. Med.* 163 (9):1009-21.

Idu D., Erhabor J.O., Efijuemue H.M. (2010): Documentation of Medicinal plants sold in markets in Abeokuta, Nigeria. *Trop. Jour. of Pharmceutical Research* 9(2):110-118.

Edema M.D. and Okiemen T. (2002). Chemical and anticonvulsant screening of *Crinum jagus. Nigeria Journal of Chemistry. Research* 7: 25-28.

Edeoga H.O., Okwu D.E., Mbaebie B.O. (2005). Phyto-chemical constituents of some Nigerian medicinal plants. *Afr. J. Biotechnol.* 4:685-688.

Faleyimu O.I., Akinyemi O., Adejoba O.R. (2009). Herbal solution to the treatment of tuberculosis infection in Kaduna, Nigeria. *Journal of Environmental Extension* 8:21-27

Fennel C.W. and Staden P. (2001). Crinum species in traditional and modern medicine. *Journal of Phyto chemistry* 5:125-131.

Furin J.J. (2007). The clinical managements of drug resistant tuberculosis. *Current Opinion in Pulmonary Medicine*: 13: 212-217.

Ghosal S., Saino K.S. and Razdans T. (1985). Crinum Alkaoids. Their chemistry and Biology. *Review Article. Phyto-chemistry* 24: 10: 2141-2156.

John R.S., Collins T., Kukundala B., Waakoc P.J. (2010). Medicinal plants used by traditional medicine practitioners in the treatment of tuberculosis. *Journal of Ethno-pharmacology*. 127:130-136.

Kumar V.A., Fausto A.K., Mutcheli N., Richard, N. (2007). In: Kumar, VA ed; Robbins Basic Pathology, Swindlers Elsevier, 8th edition USA pp 516-522.

Ode O.J., Asuzu P. (2006). The anti-snake venom activities of the methanolic extract of the bulb of *Crinum jagus*. *Toxicon* 48:331-342.

Ogunkunle A.T.J., Olopade R.O. (2011). Studies on asthma coughs plant. Crinum (Amaryllidaceac) in Nigeria. *African Journal of Plant Sciences* 5:108-114.

Peter J.H., Agbedahunsi J.M., Adegbulugbe A. (2004). Choline esterase inhibitory properties of alkaloids in *Crinum glaucum* and *Crinum jagus*. *Phyto-chemistry* 65: 2893 -2896.

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 India *J. Med. Res.* 131 :809-813.

Tram N.T.N., Titorenkova T.Z.V., Bankova V., Handjsera N.V., Popov S.S. (2002). Crinum L (Amarylidaceac): Review Fitoterapia 73, 183-208.

World Health Organisation (1993).A Global TB Deficiency. World Health Organization, Geneva, Switerzland.

Zhenkum M., Lienhardt C., Miclleron H., Nunn A., Wang X. (2010). Global tuberculosis drug development pipeline: the need and the reality. *Lancet.* 375: 2100-09