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Research Article

Anti-bacterial activity of Extract of *Crinum jagus* bulb against Isolates of *Mycobacterium tuberculosis*

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ABSTRACT: *Crinum jagus* plant has been reportedly used for treatment of infectious diseases in Nigeria. In this study, the antibacterial activity of the crude extract and chromatographic fractions from the bulb of C*rinium jagus* against *Mycobacterium tuberculosis* isolates was investigated using Lowenstein-Jensen medium (LJ) and Middlebrook 7H10 agar. Colony forming unit (cfu) was determined and percentage inhibition calculated by mean reduction in number of colonies on extract containing medium as compared to extract free control medium. The highest inhibition rate of 59% representing 56 cfu was observed for *M. tuberculosis* isolate 3 in Middlebrook 7H10 medium while similar rate of 57% was obtained on LJ medium. Fraction F3 showed 86% inhibition activity at 1.0mg/ml concentration in Middlebrook 7H10 agar compared with fractions F4 and F5 which showed 63% and 73% inhibition rates respectively. Even though, higher inhibition rates were observed with Middlebrook 7H10 agar as compared with LJ medium the difference was not statistically significant ($p>0.05$). The results support the folkloric use of *Crinum jagus* in the treatment of microbial infections and suggest that the plant may be beneficial in the treatment of tuberculosis.

Keywords: *Crinum jagus, Mycobacterium tuberculosis*, infection

INTRODUCTION

The current anti-tuberculosis (TB) drugs were discovered between 1950s and 1970s; since then there has been low activity in global TB drug research and development (R & D) until of recent. This low period of TB drug R & D has contributed greatly to the significant challenges now faced by the global community to effectively treat TB including both drug-resistant strains and TB in HIV positive individuals.

The current recommended first-line TB treatment regimens require a minimum of six months therapy, resulting in challenges with patient adherence leading to development of drug-resistant strains. It is estimated that between 5-20% of all TB cases are multidrug – resistant TB (MDR-TB; resistant to at least rifampicin and isoniazid) especially in high burden countries of Asia and Africa where public health systems are inadequate to promptly detect and treat TB (Ginsberg, 2010). Therefore, there is need for improved TB therapies. This is aimed to shorten the treatment of TB in order to improve patient adherence and prevent development of drug-resistant strains; to effectively treat drug resistant TB and also effective management of TB/HIV co-infected patients.

Medicinal plants have been used to treat infectious diseases for many years worldwide leading to a growing interest in the development of drugs of plant origin (Gupta *et al*, 2010). Nigeria is one of the countries in the world with unique wealth of medicinal plants and vast traditional knowledge of use of herbal medicine for treatment of various diseases (Olukoya *et al*, 1993; Ofukwu *et al*, 2008). The increasing incidence of MDR-TB and XDR-TB (*M. tuberculosis* isolate that is resistant to first- line drug, secondary- line drugs and quinolones and one injectable drug) globally, coupled with inadequate facilities to timely diagnose TB including drug resistant strains in high burden countries, highlight the need to search for new anti-TB drugs. Idu *et al* (2010) reported the medicinal use of roots and leaves of *Crinum jagus* for treatment of diseases of infectious origin among the local settlers

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but its anti-TB activity has not been adequately studied. This study was carried out to investigate the anti-bacterial activity of extract of bulb of the plant *Crinum jagus* against *M. tuberculosis*, the aetiological agent of TB.

MATERIALS AND METHODS

Collection of plant materials: The bulbs of *Crinum jagus* were collected from Omi-Adio, a suburb of Ibadan, Oyo state of Nigeria between March andMay, 2009. All plant specimens were identified and authenticated in the herbaria of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria.

Preparation of extracts: Fresh specimens of the plant material were chopped into pieces, air-dried and grounded into powdery form using an electrical grinding machine. About 1,127g of the powdered product was loaded into an extraction thimbe covered with cotton wool at the top. First extraction was carried out using boiling petroleum ether in soxhlet extractor apparatus (model No. 3567, Austria) for 24 hours as described by Soetan *et al*, 2006. The second extraction stage was performed using methanol as solvent. The two solvents were removed by simple distillation. The final product was transferred into a clean dry bottle, weighed and labeled as crude extract. Further extraction was carried out with the aid of flash chromatography. Separation of various fractions was achieved using thin layer chromatography method as described by Fair *et al*, 2008. Three fractions F3, F4 and F5 out of the five fractions obtained were used for anti-TB activity.

Mycobacterial isolates: Three *M. tuberculosis* isolates from three different patients identified by standard method (Barrow & Feltham, 1995) and reference susceptible strain H37Rv were collected from the TB reference laboratory, Department of Medical Microbiology, University College Hospital, Ibadan.

Assay protocol: This was performed using Lowenstein-Jensen (LJ) medium and Middlebrook 7H10 agar.

Determination of colony forming units (cfu): The ten- fold dilution of standard 1mg/ml *M. tuberculosis* suspension (Canetti *et al*, 1969) were streaked on both LJ and Middlebrook 7H10 media for determination of *cfu* in the presence or absence of plant extract. Media inoculation without plant extract served as the control. *M. tuberculosis* suspension of 1mg/ml is equivalent to MacFarland standard 1 (Kent & Kubica, 1985). One loopful (0.6ul) of this suspension was streaked on both media using 3 mm bacteriological loop. The plant extract (crude and purified) were incorporated separately on both media at concentrations of 0.2mg/ml; 0.4mg/ml; 0.6mg/ml; 0.8mg/ml and 1mg/ml of extract dissolved into 100 ml of culture medium prior to insipissation. The inoculated culture media containing extracts and the controls were incubated at 37^0C for eight weeks. Reading of the culture media was taken weekly. Percentage inhibition of each test was calculated by mean reduction in number of colonies on extract containing medium as compared to extract free control medium.

RESULTS

Of the anti TB assay using crude extract, the highest inhibition rate of 59% representing 56 cfu was observed for *M. tuberculosis* isolate 3 in Middlebrook 7H10 medium (Table 1) while similar rate of 57% was obtained on LJ medium (Table 2). There were no significant differences in percentage inhibition between the culture media

The results of the anti TB activity of chromatographic fractions of extracts of *Crinium jagus* to *M. tuberculosis* isolates are shown in Table 3 and Table 4. Fraction F3 showed 86% inhibition activity at 1.0mg/ml concentration in Middlebrook 7H10 agar compared while F4 and F5 which showed 63% and 73% inhibition rates respectively. Even though, higher inhibition rates were observed with Middlebrook 7H10 agar as compared with LJ medium the difference was not statistically significant ($p>0.05$) (Tables 3 and 4).

Table 1
Anti-TB activity of crude extract in Middlebrook 7H10 medium

Isolate	MEAN CFU AND PERCENTAGE INHIBITION RATE											
	Control		0.2mg/ml		0.4mg/ml		0.6mg/ml		0.8mg/ml		1.0mg/ml	
	CFU	% inhibition	CFU	% inhibition	CFU	% inhibition	CFU	% inhibition	CFU	% inhibition	CFU	% inhibition
H37Rv	124	0	101	19	92	26	78	37	60	52	55	56
MTB 1	120	0	108	10	92	23	78	35	64	47	54	56
MTB 2	141	0	122	14	102	27	84	40	70	50	59	58
MTB 3	138	0	116	16	98	28	84	41	68	51	56	59

Table 2
Anti-TB activity of crude extract in L-J medium

Isolate	MEAN CFU AND PERCENTAGE INHIBITION RATE											
	Control		0.2mg/ml		0.4mg/ml		0.6mg/ml		0.8mg/ml		1.0mg/ml	
	CFU	% inhibition	CFU	% inhibition	CFU	% inhibition	CFU	% inhibition	CFU	% inhibition	CFU	% inhibition
H37Rv	125	0	110	12	90	28	82	34	73	42	54	57
MTB 1	122	0	108	13	95	22	80	34	66	45	56	54
MTB 2	138	0	121	12	104	25	88	36	72	48	61	56
MTB 3	136	0	120	12	107	25	84	38	70	49	58	57

Table 3
Anti TB activity of chromatographic fractions in Middlebrook medium

Extract/ Isolate	MEAN CFU AND PERCENTAGE INHIBITION RATE											
	Control		**0.2mg/ml**		**0.4mg/ml**		**0.6mg/ml**		**0.8mg/ml**		**1.0mg/ml**	
	CFU	**% inhibition**	**CFU**	**% inhibition**	**CFU**	**% inhibition**	**CFU**	**% inhibition**	**CFU**	**inhibition**	**CFU**	**% inhibition**
F3												
H37Rv	128	0	65	49	42	67	31	76	22	83	12	91
MTB 1	158	0	104	34	94	41	82	48	65	59	37	76
MTB 2	122	0	69	43	60	51	44	64	39	68	24	80
MTB 3	154	0	78	49	68	59	52	66	38	75	22	86
F4												
H37Rv	140	0	94	32	76	46	60	52	50	64	30	79
MTB 1	131	0	112	15	98	25	90	31	72	45	58	56
MTB 2	122	0	95	22	86	29	72	41	63	48	50	59
MTB 3	136	0	102	25	95	30	77	43	68	50	50	63
F5												
H37Rv	138	0	109	21	92	33	75	46	61	56	40	71
MTB 1	126	0	94	25	78	38	62	51	48	62	40	68
MTB 2	120	0	89	26	70	42	61	49	46	62	35	71
MTB 3	140	0	96	31	78	44	65	54	48	66	38	73

Table 4
Anti TB activity of chromatographic fractions in L-J medium

Extract/ Isolate	MEAN CFU AND PERCENTAGE INHIBITION RATE											
	Control		**0.2mg/ml**		**0.4mg/ml**		**0.6mg/ml**		**0.8mg/ml**		**1.0mg/ml**	
	CFU	**% inhibition**	**CFU**	**% inhibition**	**CFU**	**% inhibition**	**CFU**	**% inhibition**	**CFU**	**inhibition**	**CFU**	**% inhibition**
F3												
H37Rv	130	0	70	46	62	52	46	65	30	77	22	83
MTB 1	164	0	102	38	96	41	86	48	69	58	44	73
MTB 2	118	0	75	36	58	51	45	62	40	66	25	79
MTB 3	162	0	82	49	70	57	58	64	40	75	28	83
F4												
H37Rv	128	0	80	38	74	42	55	23	41	68	30	77
MTB 1	128	0	108	16	102	20	98	23	76	41	64	50
MTB 2	120	0	101	16	88	18	80	33	65	46	54	55
MTB 3	138	0	116	16	110	20	89	35	73	47	58	58
F5												
H37Rv	120	0	95	21	83	31	74	38	50	58	39	68
MTB 1	120	0	90	25	74	38	64	47	52	57	42	65
MTB 2	122	0	92	25	68	44	63	48	45	63	42	66
MTB 3	142	0	98	31	76	46	67	53	52	63	44	69

DISCUSSION

Currently used anti-TB therapies are inadequate to address the many inherent and emerging challenges facing TB treatment worldwide thus; development of new medicines is a top priority of the global TB control and elimination agenda (Zhenkun, 2010).

In this study, both the crude and chromatographic fractions of extracts of *Crinium jagus* were found to inhibit growth of *M. tuberculosis* isolates. Even though there were differences in mean *cfu* and percentage inhibition rates for all the three isolates tested including the reference strain (control) using the two media, the differences were not significant (Table1-4). In spite of this, Middlebrook 7H10 agar has been reported to give a better yield of *M. tuberculosis*, requires a shorter incubation period but more expensive and with higher contamination rate than LJ medium (Sanders *et al*, 2004).

Furthermore, it was observed that chromatographic fraction 3 exhibited higher inhibition rates than the crude extract in the two media (Tables 1-4). The low inhibition rates obtained for crude extract may be due to its unpurified nature, in addition to presence of other impunities which may reduce its anti-TB potency. Of the anti-TB drugs in various stages of clinical evaluation, a diarylquiniline- based drug (TMC 207) has been found to be an inhibitor of the F0 subunit of the mycobacterial adenosine triphosphate (ATP) synthase proton pump (Andries *et al*, 2005; Koul *et al*, 2007), which is a novel mechanism of action against *M. tuberculosis* (Haagsma *et al*, 2009). This highlights the need for further studies to ascertain the active anti-TB ingredient of the *Crinium jagus* extract.

Drug resistance testing was not done on the three *M. tuberculosis* isolates used in this study due to inadequate facilities. The isolates might be MDR-TB strains. Moreover, the plant extracts were not tested along with the two most important first - line anti-TB drugs – rifampicin and isoniazid. These are some of the limitations of the study. Multidrug-resistant *M. tuberculosis* (MDR-TB) which is defined as *M. tuberculosis* isolate that is resistant to at least two most important anti-TB drugs- rifampicin and isoniazid may show more *cfu* on the media with corresponding lower inhibition rates (Gupta *et al*, 2010). Furthermore, minimum inhibitory concentration (MIC) of the extracts in suitable broth culture like Middlebook7H9 broth was not done due to financial constraints. Determination of MIC in broth culture may give more accurate result (Gupta *et al*, 2010). The use of broth culture requires expensive equipment such as Mycobacterial Growth Inhibition Tube machine (MGIT) which is not readily available in many centers in Nigeria (Kehinde *et al*, 2005). In conclusion, this study shows that chromatographic fraction 3 shows more anti-TB activity than the other two fractions including the crude extract.

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