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In vitro activity of *Bryophyllum pinnatum* and *Detarium microcarpum* plants against *Mycobacterium tuberculosis* and other bacteria

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ABSTRACT:

Two Nigerian medicinal plants *Bryophyllum pinnatum* and *Detarium microcarpum*, reputed in traditional medicine for the treatment of several ailments were selected for this investigation. The plant materials were macerated with methanol then partitioned into four fractions using solvents of different polarities. The solvent extracts were evaluated for activities against *Mycobaterium tuberculosis* (Mtb) and five other pathogens: *Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli.* The anti *Mycobaterium tuberculosis* (Mtb) assay used proportion method while agar disc diffusion method was applied for other bacteria. Hexane fractions of both plants proved very active against the test strains showing significant potency at 25µg/ml. The methylene chloride fractions of the two plant agents were also very active against *Mycobaterium tuberculosis* in a dose (25µg/ml) dependent manner compared with controls. The results revealed that the most potent fraction against *Mycobaterium tuberculosis* (Mtb) was hexane portion followed by methylene chloride layer of the two species. Also, the ethyl acetate fraction of BP possessed good antibacterial activity against the other test micro-organisms at 5mg and 2.5 mg strengths, *D. microcarpum* fractions were inactive against the bacteria. This study, in support of folkloric uses, presents *Bryophyllum pinnatum* and *Detarium microcarpum* as promising natural product agents for generating anti-infective against *Mycobaterium tuberculosis (Mtb).*

Keywords:

Antitubercular agent; Antibacterial, *Bryophyllum pinnatum; Detarium microcarpum; Mycobaterium tuberculosis*; other pathogens; Pytochemicals.

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INTRODUCTION

Tuberculosis (TB) is one of the major deadliest infectious diseases for humans and it is caused by the micro organism known as *Mycobacterium tuberculosis* (Mtb). Approximately eight million people develop this active disease every year, while 1.7 million cases of active disease result in death in the same period. Epidemiological evidence indicates that one third of the world population is infected with the causative agent, eight million cases emerge annually and about 2 million people die from this disease (Gautam *et al.,* 2007; WHO, 2007).

The rapid increase of *Tuberculosis* cases in developing countries is alarming and frequency of *M. tuberculosis* in Africa is very high as about two million new cases emerging per year (WHO, 2003). In Nigeria, like in most other developing countries, the tuberculosis situation has worsened over the past few years probably due to poor nutrition, overcrowding, lack of control measures, inadequately supervised treatment and high cost of therapy. The country was rated fifth in prevalence by WHO in its global report (WHO, 2008).

Antibiotic resistance remains a growing problem in *Tuberculosis* control programs (WHO, 2006). The situation is worsening primarily because of the association between tuberculosis and epidemic HIV/ AIDS as well as the growing prevalence of multidrug-resistant tuberculosis (MDRTB) *strains* (WHO, 2008). Standard TB drugs such as isoniazid, pyrazinamide are facing challenge of drug resistance.

So, there is an urgent need for drugs to combat the multidrug-resistant tuberculosis. Ethnobotanical survey is an important step in the identification, selection and development of therapeutic agents from medicinal plants. Antibiotics from vegetables are more investigated to combat the infections and increasing drug resistance (Anburaja *et al*., 2011 and Sriram *et al*., 2012). These growing problems have led to search for new agents of plant origin that are effective against Mtb in particular (Olugbuyiro *et al*., 2009, Suriyati *et al*., 2011). Plants have been used worldwide in traditional medicines for the treatment of various diseases. Therefore, two medicinal plants- Bryophyllum pinnatum and Detarium microcarpum were selected for this investigation.

Bryophyllum pinnatum belongs to Crassulaceae family. It has a pantropical distribution. It is a smooth, robust and unbranched herb that can grow 30-200 cm tall. It is called "Wonder of the World" in the English speaking Caribbean (McKenzie and Dunster, 1986). In ethnomedicine *Bryophyllum pinnatum* is used for the treatment of respiratory diseases. The plant is applied to induce vomiting of blood, cut umbilical cord in new borns, expel Tay Tay worms, poultries for head cold, acute and chronic bronchitis pneumonia and other forms of respiratory tract infections (Dalziel, 1936; Burkill, 1995 Some biological activities of *B.pinnatum* have been reported (Ojewole 2005; Mudi and Ibrahim, 2008). The antibacterial activity of the leaf juice of B. pinnatum was reported by Obaseiki-Ebor (1985). Flavonoids, polyphenols, and triterpenoids have been identified from the leaves of B. pinnatum (Ojewole, 2005). Quecetin-3-α-L-rhu-β- D-xyl; a flavonoid (Cao *et al*., 2005), Bryophyllin B [1], a novel potent cytotoxic bufadienolide (Yamagishi *et al*., 1989) and Malic acid (Sutton *et al*., 1972) were isolated from the leaves of *B. pinnatum.*

D. microcarpum is a tree that belongs to the family Fabaceae. The roots, stems, bark, leaves and fruits are all used to treat ailments such as tuberculosis, meningitis, itching and diarrhoea. The bark and leaves are used in dressing wounds, ulcers and fresh cuts (Dalziel, 1955). The infusion of the bark is reported to possess diuretic, anti-inflammatory and anti-parasitic properties whereas its fruits and leaves are used in the treatment of dysentery and syphilis (Iwu, 1993). Water extract of the root is used for leprosy (Collier, 2001). Species of *Detarium* have been indicated to possess antimicrobial property (Abreu *et al*., 1998; Abreu *et*

al., 1999). *Detarium sengalense* showed antiviral activity against canine parvovirus (Kudi AC, Mint, 1999) while cytotoxic (Abreu *et al*., 1999) and antibacterial properties (Ebi and Afieroho, 2011) of *D. microcarpum* have been reported. The anti- viral property of *D. microcarpum* was reported by Olugbuyiro *et al.,* (2009). Some phytochemical components of *D. microcarpum* have been reported: L-quino-1,5-lactone, D-(_)-bornesitol, D-pinitol, myo-inositol, sucrose, D-glucose, and D-fructose benzoates were isolated from the bark extract *of Detarium microcarpum* (Abreu and Relva, 2002). However, not much has been done on the antitubacular activity of these two plants. Therefore, it is the aim of this study to evaluate the potency of the medicinal plant fractions against *Mycobacterium tuberculosis* and other respiratory tract pathogens.

MATERIALS AND METHODS

Plant material

The fresh plant materials were collected from Ibadan, Oyo State, Nigeria in February 2011. The plants were identified at Forestry Research Institute of Nigeria, Ibadan, where their voucher specimens had been deposited as FHI for *Bryophyllum pinnatum* 109492 and FHI 109487 for *Detarium microcarpum*.

Extraction

Fresh leaf of *Bryophyllum pinnatum* and stem bark of *Detarium microcarpum* were used for the research. Plant cold extraction was carried out with methanol. Extract was strained and filtered over anhydrous sodium sulphate and then evaporated in vacuo at 450C. The crude extract was partitioned into hexane, dichloromethane, ethyl acetate and aqueous fractions successively. The concentrated fractions were kept in refrigerator for antimicrobial tests.

Phytochemical screening

The plant samples were phytochemically screened using standard techniques for the qualitative detection of reducing sugars, saponins, tannins, phlobatannins, steroids, cardiac glycosides, alkaloids, flavonoids and anthraquinones (Harbone, 1998; Olugbuyiro 2002, Evans, 2006).

Anti Mtb Assay

Clinical isolates and the H37Rv standard strain of *M. tuberculosis* were used and tested by the proportion method. The culture isolates and standard isolate were freshly grown on LJ medium. All clinical isolates were subjected to identification tests (Coban *et al.*, 2004) and confirmed as *M. tuberculosis.* Inoculums for the proportion method were prepared according to standard procedures (NCCLS 2002; NIMR, 2007). Each extract was diluted with acetone and adjusted in the LJ medium to final concentrations of 40 µg/ml and 25 µg/ ml. The standard drug (rifampicin) was dispensed into LJ medium at the concentration of 40 µg/ml. 0.1 ml of prepared bacterial inoculum was inoculated on LJ medium containing test drug or not drug for test or as a control and then incubated at 37°C for 28 days. Readings were taken on the 28th and 42nd days of incubation.

Resistance was defined as growth on drug containing tubes greater than 1% of the growth of drug free control medium for test extracts and RIF (NCCLS 2002; NIMR, 2007).

Antibacterial Screening

Test Organisms

Isolated strains of *Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli* were obtained from NIMR (National Institute of Medical Research) Yaba, Lagos. All the cultures were grown in Muller – Hilton agar medium.

Preparation of Inoculum

Fresh cultures of the test bacteria and the media (about 15-20 ml of Muller – Hilton agar) were poured into dishes. The test strain (0.2 ml) was inoculated into the media to inoculum size (108cells/ml) at temperature of 40-42°C. The pates were allowed to

solidify. Care was taken to ensure proper homogenization. The plant extracts were tested for antibacterial activity in the agar well diffusion assay.

Susceptibility test

The antibacterial activity was determined against methanol extracts and solvent fractions of the two plant samples. Stock solutions of 50 mg/ml and 25 mg/ml of test extracts were prepared using acetone or methanol/water (1:1) as solvent. Subsequently, 0.1ml of each concentration of the extracts was introduced into appropriate wells of 6mm diameter to afford potency of 5mg and 2.5mg respectively. The plates were incubated at 370C for 24 hours. The sensitivity test was done in replicate and the mean zone of inhibition was taken. Antimicrobial activity of the extracts was evaluated by measuring zone of inhibition and the average mean of zones were recorded.

RESULTS AND DISCUSSION

Table 1 reveals the presence of these classes of compounds: tannins, saponins, cardenolides, flavonoids and alkaloids in both *Bryophyllum pinnatum* (BP) and *Detarium microcarpum* (DM).

The activity of the extracts of *B. pinnatum* and *D. microcarpum* against *Mycobacterium tuberculosis* are displayed in **Table 2.** Hexane fractions of both plants (BP1 and DM1) proved very active against the test strains showing significant potency at 25µg/ml. Mtb was also very sensitive to the methylene chloride fractions (BP2 and DM2) in a dose dependent manner compared with controls; however, BP2 proved more active it had good sensitivity at 25µg/ml dose whereas DM2 suffered resistance at the same dose. Fractions BP3, BP4 and DM3 demonstrated good sensitivity at the concentration of 40µg/ml. The remaining plant fractions and crude extracts had no activity against *Mycobacterium tuberculosis*. The results revealed that the most potent fraction against Mtb was hexane portion followed by methylene chloride layer and that fractions of *B. pinnatum* demonstrated more significant activity as compared to *D. microcarpum*.

Tables 3 and 4 display the antibacterial profile of the test samples. While the ethyl acetate fraction of *B. pinnatum* (BP3) possessed good antibacterial activity against the test micro-organisms at 5mg and 2.5 mg strenghts, the methylene chloride and aqueous fractions of BP showed a mild activity whereas, the hexane fraction and crude extract were inactive. In Table 4, apart from the ethyl acetate fraction (DM3) which had little antibacterial activity, all other fractions and the crude extract of *D. microcarpum* were generally inactive against the test pathogens.

Table 1: Natural product classes in *Bryophyllum pinnatum* and *Detarium microcarpum*

Natural product	BP leaf	DM stem bark
Tannins	+	++
Saponins	+	++
Anthraquinones	NR	NR
Cardenolides	++	++
Flavonoids	++	++
Alkaloids	+	++

+, positive; ++, highly positive; NR, negative reaction.

Table 2: Activity of extracts of *Bryophyllum pinnatum* and *Detarium microcarpum* against *Mycobaterium tuberculosis*

Plant drug	*Sensitivity at test dose* 40µg/ml	25µg/ml
BP1	Sensitive	Sensitive
BP2	Sensitive	Sensitive
BP3	Sensitive	Resistant
BP4	Sensitive	Resistant
BP5	Resistant	Resistant
DM1	Sensitive	Sensitive
DM2	Sensitive	Resistant
DM3	Sensitive	Resistant
DM4	Resistant	Resistant
DM5	Resistant	Resistant
RIF	Sensitive	N.T

BP- *Bryophyllum pinnatum*; DM- *Detarium microcarpum*; 1-Hexane fraction 2- methylene chloride fraction; 3- Ethyl acetate fraction; 4-aqueous fraction; 5- crude extract; RIF-Rifampicin; N.T- Not Tested; Control M.TB strains: H37RV.

Table 3: Antibacterial activity of extracts of *Bryophyllum pinnatum*

Plant drug	Dose (mg)	Zone of inhibition(mm)				
		Test micro-organism				
		Staphylococcus aureus	Escherichia coli	Klebsiella pneumoniae	Pseudomonas aeruginosa	Salmonella typhi
BP1	5.0	-	-	-	-	-
	2.5	-	-	-	-	-
BP2	5.0	-	5	3	7	5
	2.5	-	5	-	5	-
BP3	5.0	17	19	20	23	20
	2.5	10	15	7	18	15
BP4	5.0	9	-	-	10	-
	2.5	-	-	-	-	-
BP5	5.0	3	-	-	-	-
	2.5	-	-	-	-	-

Our findings were in agreement with the previous antibacterial studies of *Bryophyllum pinnatum* reported (Obaseiki-ebor, 1985; Mudi and Ibrahim, 2008). The antiMtb active portions of stem bark extract of *D. microcarpum* were in active against other test bacteria. However, the antimicrobial activities of the seed coat extract have been reported by Ebi, and Afieroho (2011).

CONCLUSION

This present study demonstrates a good antimycobacterial property of the test plant samples and thus projects *Bryophyllum pinnatum* and *Detarium microcarpum* as promising natural product agents for generating anti-infective against *Mycobacterium tuberculosis*. The findings also showed that *B. pinnatum* could provide useful antibiotics against other test pathogens. This investigation justifies the use of these species in folkloric medicine. Further work is on to isolate the active principles of the plant agents.

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Table 4: Antibacterial activity of extracts of *Detarium microcarpum*

Plant drug	Dose (mg)	Zone of inhibition (mm)				
		Test micro-organism				
		Staphylococcus aureus	Escherichia coli	Klebsiella pneumoniae	Pseudomonas aeruginosa	Salmonella typhi
DMI	5.0	-	-	4	-	-
	2.5	-	-	-	-	-
DM2	5.0	-	-	-	-	-
	2.5	-	-	-	-	-
DM3	5.0	6	6	6	-	8
	2.5	-	-	-	-	-
DM4	5.0	6	-	-	6	-
	2.5	-	-	-	-	-
DM5	5.0	6	-	-	-	-
	2.5	-	-	-	-	-

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