

JOPAT Vol 22(1), 1045- 1057 Jan. – June, 2023 Edition

<https://dx.doi.org/10.4314/jopat.v22i1.3>**Synergistic activity of *Tetrapleura tetraptera* and *Abrus precatorius* fractions extract against *Streptococcus pneumoniae* and *Mycobacterium tuberculosis*****Izebe S Kasim*¹, Ibrahim YKE⁴, Onoalapo JA⁴, Ibrahim K¹, Oladosu P¹, Ibekwe N. Nneka², Adigwe P. Obi³**¹*Department of Microbiology and Biotechnology, National Institute for Pharmaceutical Research and Development, Abuja Nigeria*²*Department of Medicinal Chemistry and Quality Control, National Institute for Pharmaceutical Research and Development, Abuja, Nigeria*³*Office of the Director-General, National Institute for Pharmaceutical Research and Development, Abuja, Nigeria*⁴*Department of Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Ahmadu University Zaria, Nigeria***ABSTRACT**

The respiratory tract infections are predominantly pneumonia and are commonly associated with *Streptococcus pneumoniae* and *Mycobacterium tuberculosis* and both account for high morbidity and mortality globally. This study is aimed to determine the antimicrobial and synergistic activity of *Tetrapleura tetraptera* and *Abrus precatorius* against these two respiratory infections. The leaves of *T. tetraptera* and *A. precatorius* were extracted using 70% methanol and further fractionations of the further fractionation. The crude and fractions were screened for antimicrobial activity on *Streptococcus pneumoniae* and *Mycobacterium tuberculosis* using micro-dilution technique. The synergistic activity of fractions of *T. tetraptera* and *A. precatorius* was analyzed using standard checkerboard. The phytochemical composition of the fractions was analyzed using standard technique. The crude extracts of *T. tetraptera* and *A. precatorius* had activity against *S. pneumoniae* and *M. tuberculosis* at varied concentrations of 0.7 to 6.9 mg/l. The fractions alone of *T. tetraptera* and *A. precatorius* had activity at lower concentrations ranging 0.03 to 0.98 mg/ml against test bacteria. The combined fractions *T. tetraptera* and *A. precatorius* against *S. pneumoniae* and *M. tuberculosis* provided better activity at concentrations of 0.01 to 0.33 mg/ml to give synergy and indifference activity. The phytochemicals identified in *A. precatorius* and *T. tetraptera* included tannins, saponins, flavonoids, phenols and resins but differ in alkaloids detected in only *T. tetraptera*. This study has shown that *A. precatorius* and *T. tetraptera* possess antimicrobial activity against *Streptococcus pneumoniae* and *Mycobacterium tuberculosis*. The combined fractions of *T. tetraptera* and *A. precatorius* showed greater antimicrobial activity than when each plant is used individually. The synergistic effect of these fractions of *T. tetraptera* and *A. precatorius* can be used in the development of phyto-medicine that will be efficacious, increase cure rate and decrease resistance in *S. pneumoniae* and *M. tuberculosis*.

Keywords: *Abrus precatorius*, *Tetrapleura tetraptera* crude extract, fractions, synergistic, micro-dilution, *Streptococcus pneumoniae*, *Mycobacterium tuberculosis*, pneumonia

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INTRODUCTION

Respiratory tract infections are associated with prominently with *Streptococcus pneumoniae* and *Mycobacterium tuberculosis*. The disease state includes bronchiolitis in young, elderly and pneumonia in all ages accounted for 2.5 million deaths in 2019.

[1] Notably, pneumonia ranks among the top 10 conditions with respect to the cost of hospitalization [2] and has been found to be 0.22 times per child per year in developing countries [3]. Tuberculosis (TB) has high morbidity and mortality globally with two third of world population got sick and 1.5 million deaths recorded in 2019. [3] *S. pneumoniae* and *M. tuberculosis* can enhance diseases state with low immunity of individual in cases of immunodeficiency virus and use of suppressant drugs, diabetes, smoking, alcohol and drug use [4]. A recent study has shown that in community acquired pneumonia patients with *Streptococcus pneumoniae* and *M. tuberculosis* co-infection, pneumococcal pneumonia is likely to provide fertile ground for reactivation of latent TB [5] In patients with multidrug-resistant TB (MDR-TB), long-term use of medication increases the risk of secondary pneumococcal pneumonia [7]. In patients with MDR-TB, long-term use of medication increases the risk of secondary pneumonia. In turn, secondary pneumonia significantly delays in smear conversion to

negative in patients with MDR-TB. This is also a cause to TB treatment failure [6]. Pneumonia and TB can be combined or exist one after another, and this would cause certain difficulties in diagnosis of the two diseases [7]. The emergence of resistance strains of *S. pneumoniae* to conventional drugs have led to finding more active drugs [8]. Center for Disease Control [42] reported 4.1% and 2.1.1% of *S. pneumoniae* isolates were resistance to penicillin and cefotaxime respectively. The MDR-TB is defined as resistance to rifampicin, isoniazid and second line injectable drugs [3]. *S. pneumoniae* and *M. tuberculosis* have made use of pneumococcal and BCG vaccination respectively ineffective in the control and management of pneumonia and the concerned of emerging resistance vaccines and convectional drugs. Combination therapy for TB treatment is to prevent drug resistance, improve cure and shorten treatment [40]. The global multidrug-TB has over 500,000 cases despite combination therapy [43]. *S. pneumoniae* associated pneumonia is treated with combined drugs of amoxicillin-Clavulanate. Ethno medicine surveys of have been done and documented for medicinal uses as reported by researchers [35] [34], for the management of most ailments. *Abrus precatorius* is an indigenous plant with array of medicinal uses. *Abrus precatorius* (Idon Zakara in Hausa), Oju Ologbo in Yoruba) possess antimicrobial activity. [10] [27] [18]

[33]. *Tetrapleura tetraptera* (Dawo in Hausa), (Uyayak in Igbo), (Aridan/Aidan in Yoruba) and Ighimiakia in Bini) has antimicrobial activity. [38] [36] [25] [13] *Tetrapleura tetraptera* possess anti-tuberculosis activity. [37] The phytochemical compositions of the *T. tetraptera* Adeleye et al [31] Ogugor et al [39] and *A. precatorius* [27] [28] [30] have been reported and responsible for the antimicrobial activity. The checkerboard method is a traditional method that tests synergy between two or more drug combinations. It reflects the bacterial growth inhibition based on MIC results, by using an equation to calculate the fractional inhibitory concentration index (FICI) as reported by Fadwa et al [15] and Orhan et al [16]. The study is aimed at determining the phytochemical composition, antimicrobial and synergistic activity of *Tetrapleura tetraptera* and *Abrus precatorius* on *Streptococcus pneumoniae* and *Mycobacterium tuberculosis* associated with pneumonia.

MATERIALS AND METHODS

Clinical isolates of *Streptococcus pneumoniae* and *Mycobacterium tuberculosis* and BCG strains (ATCC 35737) were obtained from Department of Microbiology and Biotechnology, National Institute for Pharmaceutical Research and Development (NIPRD) and were authenticated with quality culture media. All

reagents used for phytochemicals screening were of analytical grade.

Plant collection and identification

The leaves of *A. precatorius* were collected from Suleja environs, Niger State, Nigeria while leaves of *T. tetraptera* were obtained from Ihievbe, Owan East environs, Edo State, Nigeria. Specimens of both plants with voucher numbers NIPRD/H/6795 and NIPRD/H/6808 respectively were identified in Herbarium Unit, National Institute for Pharmaceutical Research & Development, Abuja. The plant materials were air-dried, pulverized into powder and stored at room temperature until ready for use.

Preparation of crude extract

One hundred and sixty (160) grams of pulverized leaf materials was transferred into 2.0 L of 70% methanol flask and allowed to soak for 24 hours, filtered and filtrate was concentrated using rotator evaporator technique. The extract deposit was scrapped into cleaned sterile container and stored at 4°C until ready for use.

Preparation of fractions

Methanol extract of the plant was used for the fractionation procedure. 10.0 g of extracts was weighed and mixed with 10.0 g silica gel and allowed the methanol to evaporate. 30 g of silica gel of 60G mesh and chromatographed on column of silica gel – 60. Slurry packed in

petroleum ether. The column was gradient eluted first with n – hexane and then with acetyl acetate and finally with methanol. The solvent system included 100ml of hexane to elute followed with decrease volume of 100, 80, 60, 40, and 20 ml. This was followed with ethyl acetate in the order of 20, 40, 60, 80 and 20 ml to elute the system. [22, 17].

Preparation of test microorganisms

The overnight culture of *S. pneumoniae* was sub-cultured into fresh broth and incubated for three (3) hours and standardized to 0.5 McFarland solution. Two weeks' cultures of clinical isolates of *M. tuberculosis* and *BCG* strains in Middlebrook 7H9 enriched with albumin dextrose catalase were authenticated for acid fast bacilli using Zeihl-Neelsen technique. The population of *M. tuberculosis* and *BCG* was standardized to 0.5 McFarland

Minimum inhibitory concentration

In sterile 96 well micro-plates a two-fold dilutions of the crude extract were made to obtain the desired starting concentration. 50µl of sterile Middle Brook 7H9 broth was dispensed into the 96 labeled micro well plates excluding the first row each of the eight rolls. 100µl of diluted extract was dispensed onto the first row of the 96 micro well plates. Two fold dilutions were carried out using 50µl broth of each of the 1stwell and dispense onto the 2nd well and

continue to the 9th. The 10th well contains 100µl of the extract concentration (extract sterility), 11th well contain 100µl of media (media sterility control) and the 12th well contained the organism viability control. The procedure was repeated in triplicate. The 96-micro-plate was each inoculated with each test microorganism and labeled. All inoculated plates were incubated at 37^oC for 24 hours for *S. pneumoniae* and 5 to 7 days for *M. tuberculosis*. At the end 24 hour/ 5days, 25µl of tetrazolium salt was added to wells to determine color change indicative of growth and no activity, while no color change in the controlled labeled wells indicate further incubation for 48 hours. At end of 48 hours 25µl tetrazolium was further dispensed into controlled wells for activity by change in color. The absent of color change showed inhibition of test microorganism (activity) and present of color showed no inhibition or growth of test microorganisms. The highest dilution that inhibits the growth of microorganisms was the minimum inhibitory concentration of the plant extract. The above procedure was carried out for the different plant extracts and on the different test microorganisms. The above procedure was repeated for the fractions of *T. tetraptera* and *A. precatorius* [19, 29 and 24].

Minimum bactericidal concentration determination

The results from the minimum inhibition concentration (MIC) plates were used for the minimum bactericidal concentration (MBC) determination. In the wells that did not show growth by means of no color change, 100µl each was inoculated into fresh sterile Muller Hinton agar and incubated at 37°C for 24 hours and count the number of colony and compared with the initial growth per ml (10^5 cfu/mL). for *S. pneumoniae*. The initial population *M. tuberculosis* strain was cultured in Middlebrook 7H11 agar enriched with oleic acid albumin dextrose catalase and incubated at 37°C in 10% CO₂ for 7 to 14 days and gave 10^3 colony forming unit (CFU) count. The wells that exhibited activity in the MIC test were cultured in Middlebrook 7H11 and growth compared with initial population of *M. tuberculosis* in the crude extract or fractions with average of 10^2 CFU/ ml. The minimum bactericidal concentration was computed if the growth was less than 0.1% [20] [24].

Qualitative Phytochemistry determination

The crude methanol extracts of *A. precatorius* and *T. tetraptera* were qualitatively assessed for saponins, tannins, flavonoids, phenols, steroids/ terpenes, alkaloids, anthraquinones and glycosides; this was carried out following standard procedures [9] [23] [17].

Checkerboard assay for Synergistic Activity

The fractions of *T. tetraptera* and *A. precatorius*, 0.10g each was weighed and dissolved in sterile distilled water. Twice MIC concentration of *T. tetraptera* (A) and *A. precatorius* (B) were prepared followed two fold dilutions according to National Committee for Clinical Laboratory Standards (NCCLS) recommendation prior to testing. [40] 50.0µl of Middlebrook 7H9 broth was distributed into each well of the micro-dilution plates. 50.0µl of A was serially dispensed along the ordinate column 8 down to row G. 50.0µl of B was serially diluted along the abscissa from row H well 1 to 7th well. The dilutions of A and B were systemically combined from row A well to well 7. 25 µl of serial dilutions of *T. tetraptera* and *A. precatorius* were combined to fill the wells. 50 µl of *M. tuberculosis* culture compared with 0.5 McFarland turbidity (5.0×10^5 CFU/ml) were prepared and dispensed into all the wells. The controls for positive drug sterility, media sterility and organism viability wells were included. The above was replicated with BCG. The inoculated well plates were incubated at 37°C for 24 to 48 hours for *S. pneumoniae* and 7 days for *M. tuberculosis* with daily observation. At the end of incubation period, 25.0µl tetrazolium solution added to the wells to detect inhibition or growth by colour change. [16][15]

RESULTS AND DISCUSSION

Table 1: Qualitative screening of phytochemical in *T. tetraptera* and *A. precatorius*

Parameters	<i>Tetrapleura tetraptera</i>	<i>Abrus precatorius</i>
Tannins	+	+
Alkaloids	+	-
Saponins	++	+
Flavonoids	+	+
Phenols	+	+
Terpenes	-	-
Steroids	-	-
Glycosides	-	-
Resins	+	+
Anthraquinones	-	-

Key: (+) presence (-) absent

Table 2: Antimicrobial determination of *A. precatorius* and *T. tetraptera* against *S. pneumoniae* and *M. tuberculosis*

Plant Crude	<i>S. pneumoniae</i> (mg/ml)		<i>M. tuberculosis</i> (mg/ml)		BCG (mg/ml)	
	MIC	MBC	MIC	MBC	MIC	MBC
<i>A. precatorius</i>	0.7	0.14	1.4	2.8	5.6	11.5
<i>T. tetraptera</i>	0.9	1.8	6.9	13.8	6.9	13.8
Control (RIF)	0.004		0.004		0.004	

Key: MIC=Minimum inhibitory concentration, RIF= Rifampicin

Table 3: Antimicrobial activity of fractions of *T. tetraptera* and *A. precatorius* on *S. pneumoniae* and *M. tuberculosis*

Fractions	<i>Streptococcus pneumoniae</i> (mg/ml)		<i>Mycobacterium tuberculosis</i> (mg/ml)		BCG (mg/ml)		
	MIC	MBC	MIC	MBC	MIC	MBC	
<i>A. precatorius</i>	3	0.45	0.9	NI	ND	NI	ND
	4	0.49	0.98	0.98	1.96	0.98	1.96
	6	1.11	2.22	NI	ND	NI	ND
<i>T. tetraptera</i>	7	0.49	0.99	0.49	0.99	0.49	0.99
	8	0.24	0.48	0.3	0.6	0.3	0.6
	11	0.03	0.05	0.11	0.22	0.11	0.22
Control (RIF)	0.004		0.004		0.004		

Key: MIC= Minimum inhibitory concentration, MBC= Minimum bactericidal concentration, NI= No inhibition, ND= Not done, RIF=Rifampicin

Table 4: Synergistic screening of fractions *T. tetraptera* and *A. precatorius* fraction

Fraction combination	<i>Streptococcus pneumoniae</i>		<i>Mycobacterium tuberculosis</i>	
	ΣFIC	Activity	ΣFIC	Activity
T7 - A4	0.50	S	0.50	S
T8 - A4	0.49	S	0.53	I
T11 - A4	0.51	I	0.53	I

Key: T = *Tetrapleura*, A= *Abrus*, FIC= Fractional inhibitory concentration, S= Synergy, I= Indifference

The methanolic extraction of leaves of *A. precatorius* and *T. tetraptera* yielded 3.7% and 2.6% respectively. The phytochemical screening for *A. precatorius* and *T. tetraptera* revealed the presence of tannins, saponins, flavonoids, phenols, resins but differ in alkaloids present only in *T. tetraptera* (Table 1). This study finding agrees with others researchers on the phytochemical identified in *T. tetraptera*. [31][39] Enema et al [32] reported presence of glycoside that differs from the findings of this study. The absence of alkaloids in this study differ from documented works that revealed alkaloids in *A. precatorius* [21] [28] [30]. The concentration of *T tetraptera* and *A. precatorius* crude extracts against *S. pneumoniae* and *M. tuberculosis* (Table 2). The crude of *A. precatorius* at the concentrations of 0.7, 1.4 and 2.6 mg/ml inhibited of the growths of *S. pneumoniae*, *M. tuberculosis* and *BCG* respectively, and a bactericidal action at concentrations of 1.4, 2.8 and 5.2 mg/ml against *S. pneumoniae*, *M. tuberculosis* and *BCG* respectively. This study agrees with works of

Adelowotan et al [10] that reported 0512 mg/ml of *A. precatorius* against clinical *S. milleri*. A similar study by Misrahanum et al [11] reported higher concentration of 75 mg/ml inhibited the growth of *S. pneumoniae*, Adnan et al [12] reported the use of *A. precatorius* traditionally for management of pneumonia with concentration of 200mg/ml. Mann et al [27] reported no activity of methanol extraction of *A. precatorius* against *BCG* at concentration of 0.032 mg/ml which differ from 5.6 mg/ml of *A. precatorius*. *T. tetraptera* crude extracts at concentrations of 0.9, 6.9 and 6.9 mg/ml inhibited the growth of *S. pneumoniae*, *M. tuberculosis* and *BCG* respectively, while at concentrations of 1.8, 13.8 and 13.8 mg/ml had bactericidal action on *S. pneumoniae*, *M. tuberculosis* and *BCG* respectively. A previous study, Koma et al [14] reported minimum inhibition concentration (MIC) of 0.625 mg/ml of crude leaf extract of *T. tetraptera* against *S. pneumoniae* which agrees with findings of this study. The fractions of *A. precatorius* 3, 4, and 6 at concentrations of 0.45, 0.49 and 1.11 mg/ml

inhibited the growth of *S. pneumoniae* similarly had bactericidal action at concentrations of 0.90, 0.98 and 2.22 mg/ml against *S. pneumoniae* (Table 3). Okar *et al* [33] reported activity of fractions of *A. precatorius* at concentrations 20 to 30 μ M against multidrug resistant *S. aureus*. The fractions 3 and 6 of *A. precatorius* had no inhibitory activity against *M. tuberculosis* and *BCG*. However, *A. precatorius* fraction 4 concentrations of 0.98 and 0.98 mg/ml had inhibitory action against *M. tuberculosis* and *BCG* respectively. In a similar study, Ibekwe *et al* [18] reported inhibition of *M. tuberculosis* and *BCG* at concentrations of 0.50 and 1.84 mg.ml respectively. The fractions 7, 8 and 11 of *T. tetraptera* each combined with fraction 4 of *A. precatorius* were screened against *S. pneumoniae*, *M. tuberculosis* (Table 4). The checkerboard microliters plate assay is used to test the activities of several drugs in combination against *S. pneumoniae* and *M. tuberculosis* strains by determining the Σ FICs of all combinations tested. The combinations of fraction of *T. tetraptera* (T7) and fraction of *A. precatorius* (A4) against *S. pneumoniae* gave combined fractional inhibitory concentration (Σ FIC = 0.48; Σ FIC = MIC T7 + MIC A4/MIC T7) which is less than 0.5 thus, this combination is synergy. Similarly, combination of fractions of *T. tetraptera* (T8) and *A. precatorius* (A4) revealed synergy (FIC=0.49) against *S. pneumoniae*. The combination of fractions *T.*

tetraptera (T8) and *A. precatorius* (A4) revealed indifference action (FIC=0.57). The combination of fractions of *T. tetraptera* (T7) and *A. precatorius* (A4) against *M. tuberculosis* revealed synergy action (FIC=0.50), while other combination of *T. tetraptera* (T8) and *A. precatorius* (A4) and *T. tetraptera* (T11) and *A. precatorius* (A4) against *M. tuberculosis* revealed indifference activity (FICs 0.53 and 0.53) respectively. There are no similar works been reported in literature on synergistic activity of *T. tetraptera* and *A. precatorius* in recent times. The use of checkerboard assay in the efficacy of combined therapy has compared with other known methods [16] [15].

CONCLUSION

The potential of *T. tetraptera* and *A. precatorius* fractions has revealed inhibitory activity against *Streptococcus pneumoniae* and *Mycobacterium tuberculosis* as a result of the presence of phytochemical. The antimicrobial activity of individual plant is low in activity against *S. pneumoniae* and *M. tuberculosis*, and when combined revealed higher antimicrobial activity. The combinations of these plants act complementary effect in the antimicrobial activity. The synergy and indifference activity of fractions of these plants have better advantage in drug development. The combination of *T. tetraptera* and *A. precatorius* that yield synergy can be a potent ingredient for a phyto-medicine

that is more efficacious, with goal of shorten treatment, increase cure rate and decrease drug resistance of *S pneumoniae* and *M. tuberculosis*.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will borne by them.

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