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Inhibitory activities of *Ceiba pentandra* (L.) Gaertn. and *Cordia sebestena* Linn. on selected rapidly growing mycobacteria

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The plants *Ceiba pentandra* (L.) Gaertn. [Malvaceae previously Bombacaceae] and *Cordia sebestena* Linn. [Boraginaceae] used in this study were selected to investigate and justify their local use in the treatment of cough, catarrh, sore throat, pneumonia and tuberculosis, all of which are associated with respiratory tract infections. The antimycobacterial activities of these plants were investigated in *Mycobacterium fortuitum* ATCC 684, *Mycobacterium smegmatis* ATCC 19420, *Mycobacterium abscessus* and *Mycobacterium phlei* ATCC 19240. The agar cup diffusion method was used for the antimycobacterial screening at concentrations of 10, 20, 100 and 200 mg/ml while the agar dilution method was used for the determination of the minimum inhibitory concentration (MIC). Phytochemical screening revealed the presence of tannins, cardenolides, alkaloids, anthraquinones and saponins in all the plant samples except in *C. sebestena* leaf in which saponins and anthraquinones were absent. The inhibitory activity of methanolic extracts of the stem barks of *C. pentandra* and *C. sebestena* on the test organisms was dose-dependent. The MIC and the minimum bactericidal concentration (MBC) of the extracts ranged from 20 - 200 and 40 - 600 mg/ml, respectively. The results obtained in this study justify the ethnomedicinal use of the plants in conditions associated with respiratory tract infections.

Key words: Inhibitory activities, *Ceiba pentandra* (L.) Gaertn., *Cordia sebestena* Linn., rapidly growing mycobacteria, *in vitro.*

INTRODUCTION

Ceiba is the name of a genus of about 17 species in the family Malvaceae, native to Mexico, Central America and the Caribbean, northern South America and tropical West Africa (Gibbs and Semir, 2003). The best-known, and most widely cultivated, species is Kapok, *Ceiba pentandra*. Other species of *Ceiba* include *Ceiba aesculifolia, Ceiba trichistandra, Ceiba chodati* and *Ceiba speciosa*. In the Kano area of Northern Nigeria, they are

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pounded to a fine state to apply as a curative dressing on sores. Pounded leaves are applied as a dressing on sores, tumours, abscesses and whitlows (Burkill and Dalziel, 1997). Leaf infusion is taken orally against cough and hoarse throat. Stem and bark are used as antibacterial, heart tonic and in kidney stone, headache and snake bite. In Nigeria, the bark is used on skin infections, and the bark infusion is taken as a febrifuge (Burkill, 2000). The root is used in various remedies for leprosy in Ivory Coast-Upper Volta (Burkill and Dalziel, 1997). Young leaves are a source of calcium and iron. Seeds contain oil, 24.2% ash, 5.22% crude fiber, 23.9% albuminoids, 18.9% carbos and others, 15.9%. The oil is a mixture of fatty acid, 70% liquid, 30% solid palmitic acid. It also contains fatty acids glycosides, saponins and steroids (Sarkiyayi et al., 2009). Chemicals identified in the floss are pentosans and uronic anhydrides (Burkill and Dalziel, 1997).

Cordia is a genus of shrubs and trees in the borage family Boraginaceae. About 300 species have been identified worldwide, mostly in warmer regions. Many of the species are commonly called manjack while bocote may refer to several Central American species in Spanish (Quattrocchi, 2000). The genus *Cordia* is a known source of benzoquinones, naphthoquinones, hydroquinones, cromenes, triterpenes, sesquiterpenes, polyphenols and flavonoids. Many compounds originally isolated from *Cordia* species have been reported as presenting several biological activities such as antifungal, larvicidal, anti-inflammatory and anti-androgenic (Renata et al., 2005). Syrup of the bark, flowers, or fruit is taken for coughs and bronchial ailments. The tree's sap is applied to wounds. Four phenylpropanoid esters, sebestenoids A-D have been isolated from the fruit of *Cordia sebestena* (Dai et al., 2010). The chemical compounds: robinin, rutin, datiscoside, hesperidin, dehydrorobinetin, chlorogenic acid and caffeic acid have been isolated from *Cordia francisci, Cordia myxa* and *Cordia serratifolia* (Wiart, 2006). The aim of this study is to evaluate and justify the folklore use of *C. sebestena* (L.) Gaertn. and *C. pentandra* Linn. in the treatment of coughs and bronchial ailments associated with lower respiratory tract infections. Rapidly growing mycobacteria similar genotypically but more resistant than *Mycobacterium tuberculosis* were used in this study to evaluate the antimycobacterial potentials of the test plants because they are less pathogenic than *M. tuberculosis* which is actually the main target for developing new antimycobacterial agents.

MATERIALS AND METHODS

Plant collection and identification

Fresh stem bark and leaves of *C. pentandra* (L.) Gaertn. and *C. sebestena* Linn. were collected from University of Ibadan, Oyo State, Nigeria. The plants *C. pentandra* and *C. sebestena* were identified by Mr. Donaltus, a herbarium officer of the Department of Botany, University of Ibadan. The plants *C. pentandra* and *C. sebestena* were authenticated at Forestry Research Institute of Nigeria (FRIN), Jericho, Ibadan, Nigeria and assigned Voucher specimen numbers FHI 108935 and FHI 108934, respectively. The plant parts were air-dried, pulverized, weighed and stored for the study.

Phytochemical screening

The leaf and stem bark of *C. pentandra* and *C. sebestena* were screened for the presence of secondary metabolites such as alkaloids, cardenolides, saponins, tannins and anthraquinone glycosides using the methods described by Harborne (1998).

Extraction procedure and preparation of extracts

Extracts of the test plant samples were obtained by cold extraction at room temperature. Pulverized *C. pentandra* leaves (304.8 g), *C. pentandra* stem bark (1105.7 g), *C. sebestena* leaves (288.1 g) and *C. sebestena* stem bark (661.9 g) were soaked in *n*-hexane for 48-72 h after which the solvent was decanted. The samples were air-dried and soaked in methanol for about 48 h with constant agitation after which the solvent was decanted and more solvent added successively to ensure complete extraction. The methanol extract was concentrated *in vacuo*, weighed and stored for further studies. The crude methanol extracts was partitioned into dichloromethane. The methanol and dichloromethane fractions were collected, concentrated, dried and weighed. Four different concentrations were prepared for each of the crude extract used, and they were 10, 20, 100 and 200 mg/mL. Extracts were reconstituted in 20% dimethylsulfoxide (DMSO).

Antimicrobial screening of plant materials

All the test microorganisms were investigated for their susceptibilities to the various plant extracts by means of agar diffusion technique (Hugo and Russel,1998; Adeniyi et al., 2006, Lawal et al., 2011). The following microorganisms were used in the study: *Mycobacterium fortuitum* ATCC 684*, Mycobacterium smegmatis* ATCC 19420*, Mycobacterium abscessus, and Mycobacterium phlei ATCC* 19240. Overnight bacterial cultures in Tryptic soy broth (Oxoid, UK) were obtained by subculturing from the stored slopes. A 1 in 100 overnight broth culture of each bacterium in appropriate broth medium was made by adding 0.1 ml of the broth into a 9.9 ml of sterile distilled water. Using a sterile pipette, 0.2 ml of the 1 in 100 dilution overnight culture of the test organism was seeded into 20 ml of melted and cooled (45°C) Mueller-Hinton agar (Oxoid, UK) supplemented with 10% blood, poured into sterile Petri-dishes after thorough mixing between palms and allowed to set. The surfaces of the plates were dried in an already disinfected oven at 37°C in an inverted position. Equidistant wells were bored into the solidified medium using a sterilized cork borer of diameter 8 mm. One hundred microlitre (100 µl) volumes of each reconstituted extract at concentrations 10, 20, 100 and 200 mg/ml were introduced into the wells. 10 and 20 µg/ml concentrations of Rifampicin were introduced into two of the wells to serve as positive control while DMSO (20%) was used as the negative control. The plates were left

Table 1. Yield and macroscopic characteristics of extracts of *Ceiba pentandra* (L.) Gaertn. and *Cordia sebestena* Linn.

Plant	Plant part	Weight of ground sample (g)	Yield (g)		Yield (%)		Macroscopic characteristic	
			MeOH	Dichloro.	MeOH	Dichloro.	MeOH	Dichloro.
Ceibapentandra	Leaf	304.8	8.3	4.0	2.7	1.3	Shiny dark-brown congealed extract	Greyish congealed extract
	Stem bark	1105.7	12.1	2.2	1.1	0.2	Shiny reddish-brown and slightly powdery extract.	Dark-brown congealed extract
Cordiasebestena	Leaf	288.1	26.7	7.6	9.3	2.7	Dark-brown congealed extract	Greyish congealed extract
	Stem bark	661.9	29.4	2.8	4.4	0.4	Shiny dark-brown congealed extract.	Shiny reddish-brown extract.

MeOH- Methanol; Dichloro- dichloromethane.

for one hour at room temperature to allow pre-diffusion of the extracts and controls in the well through the agar after which they were incubated at 37°C for 24 h. After incubation, the zones of diameter of inhibition were measured and recorded. All tests were performed in duplicates.

Determination of minimum inhibitory concentration (MIC)

The MIC for the bioactive extracts was determined by the agar dilution procedure guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2008). Different concentrations of the extracts were prepared to final concentration in the range of 200 to 25 mg/ml in 20% DMSO. One millilitre (1 ml) of the extract from each dilution was mixed with 19 ml of melted and cooled (45°C) in Mueller-Hinton agar (Oxoid, UK) was supplemented with 10% blood and poured into sterile Petri dishes allowing the agar to set. The surface of the agar was allowed to dry before streaking with overnight broth cultures of test organisms. The plates were incubated at 37°C for 24 h and examined for the presence or absence of growth. The lowest concentration preventing visible growth was taken as the MIC of the extract. All procedure was performed in duplicates.

Determination of minimum bactericidal concentration (MBC)

The MBC for the bioactive extracts were determined by a modification of the method of Aibinu et al. (2007). Concentrations higher than and equivalent to the MIC were prepared in Tryptic Soy broth (Oxoid, UK), 0.5 mL of a 24 h culture of test organisms were added to 4.5 ml of the extracts solution in test tube. The mixture was incubated at 37°C for 24 h after which aliquots of samples were withdrawn. Ten-fold dilutions were made and 0.2 mL of 10^{-3} dilution was transferred onto extract-free sterile melted and cooled (45°C) in Mueller-Hinton agar (Oxoid, UK) supplemented with 10% blood in Petri dish. The agar plates were incubated at 37°C for 24 h and observed for absence or presence of growth. The minimum concentration preventing visible growth of the organisms was taken as MBC. All procedure was performed in duplicates.

RESULTS AND DISCUSSION

The extraction yield, percentage yield and macroscopic characteristics of the plants extracts are presented in Table 1. The yield in percentage of the methanol extracts of the plant samples was about 5 times more as compared to the dichloromethane extract. This, however, is not in agreement with the report of Cowan (1999) that ranked methanol second next to methylene dichloride (dichloromethane) in terms of yield in extraction of plant active components. This may be due to the fact that the dichloromethane extract was obtained from partitioning of the methanol extracts. The higher yield values of the methanol extracts may suggest that polar compounds abound in the plant samples since methanol is a more polar solvent relative to dichloromethane. Phytochemical screening of the plant samples revealed the presence of tannins, cardenolides and alkaloids in all the plant samples while anthraquinones and saponins were present in all samples except *C. sebestena* leaf (Table 2). These phytochemical compounds are known to be biologically active and thus aid the antimicrobial activities of *C. pentandra* and *C. sebestena.* Phytochemicals exert antimicrobial activity through different mechanisms; tannins for example, act by iron deprivation, hydrogen bonding or specific interactions with vital proteins such as enzymes (Scalbert, 1991) in microbial cells. Tannins are known to provide the typical tanning effect which is important for the treatment of inflamed or ulcerative tissues (Parekh and Chanda, 2007). Herbs that have tannins as their component are astringent in nature and are used for treating intestinal disorders such as diarrhoea and dysentery (Dharmananda, 2003) thus

Table 2. Phytochemical screening of *Ceiba pentandra* (L.) Gaertn. and *Cordia sebestena* Linn.

Phytochemical grouping	Plant/morphological parts			
	***Ceiba* leaf**	***Ceiba* stem bark**	***Cordia* leaf**	***Cordia* stem bark**
Anthraquinones	+	+	-	+
Tannins	+	+	+	+
Saponins	+	+	-	+
Cardenolides	++	++	+	++
Alkaloids	+++	+++	+	+

+ = Low concentration; ++ = moderate concentration; +++ = high concentration.

Table 3. Antimycobacterial screening of *Ceiba pentandra* (L.) Gaertn leaf extracts. Mean diameter of zone of inhibition (mm) ± SEM.

Extract (mg/mL)	Methanol (mg/mL)				Dichloromethane (mg/mL)				RMP (µg/mL)		DMSO
	10	20	100	200	10	20	100	200	10	20	(20%)
M. fortuitum ATCC 684	-	-	12±0.0	12±0.0	-	-	10±0.0	11±0.0	25±0.0	27±0.0	-
M. smegmatis ATCC 19420	-	-	12±0.0	14.5±0.5	-	10±1.5	12±0.0	12±0.0	28±0.0	29±0.5	-
M. abscessus	-	-	-	-	-	-	-	-	20±0.0	20±0.0	-
M. phlei ATCC 19240	-	-	-	-	-	-	-	-	20±0.0	22±0.5	-

Diameter of cork borer = 8 mm, - = resistance, RMP = Rifampicin.

exhibiting antimicrobial activity. The presence of tannins in *C. pentandra* and *C. sebestena* supports the traditional medicinal use of these plants in the treatment of different ailments. Li et al. (2003) reviewed the biological activities of tannins and observed that tannins have remarkable activity in cancer prevention and anticancer, thus suggesting that the plants have potentials as a source of important bioactive molecules for the treatment and prevention of cancer. Moreover, ethnobotany of *C. pentandra* claims that its pounded leaves are applied as a dressing on tumours (Burkill and Dalziel, 1997). In addition to its antimicrobial and anticancer activities, tannins have roles such as stable and potent antioxidants. Tannins have been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional proteins unavailable for them (Sodipo et al., 1991). Alkaloids are compounds needed for cell activity and gene code in the genotype. They are biologically significant as active stimulator and terminators of growth, a part of endogenous security and mechanism. They display antimicrobial and anti-parasitic properties; they are non toxic to the plants that produce them. Biotoxicity is directed only towards foreign organisms or cells and it is selective. Alkaloids can alter DNA and selectively deform cells (Carl, 2007). These biological activities of alkaloids may also have contributed to the the antimycobacterial activities of *C. pentandra* and *C. sebestena.*

The results of the antimicrobial screening of the extracts are presented in Tables 3 to 6 which showed that the methanol extracts of both plants were the most active against the test microorganisms. The activity demonstrated by the fractions was dose-dependent as shown by the diameter of zone of inhibition for most of the active extracts. The diameter of the zone of inhibition for most of the extracts increased as the concentration of the extracts increased. Antimycobacterial screening showed that the test organisms had varied susceptibilities to the extracts, the most susceptible being *M. fortuitum* ATCC 684 while the most resistant was *M. abscessus* (Tables 3 to 6). The most active of the extracts against all the test organisms was *C. pentandra* stem bark. The test organisms also demonstrated varied susceptibility to the control drugs as shown in Tables 3 to 6. From the results obtained in this study, it was observed that the plant extracts demonstrated antimicrobial activities against the selected organisms. This was evidenced by the varying zones of inhibition of the individual extracts on the test organisms. However, 10 mg/ml of the methanol and the dichloromethane leaf extracts of the two plants showed no activity against all the test organisms. The results of the MIC and MBC of the bioactive extracts are shown in Table 7. The MIC value for the bioactive extracts against the bacteria was 20 - 200 mg/ml. *C. pentandra* stem bark methanol extract produced the lowest MIC value of 20 mg/ml for some of the bacteria. This suggests that this

Table 4. Antimycobacterial screening of *Ceiba pentandra* (L.) Gaertn. stem bark extracts. Mean diameter of zone of inhibition (mm) ± SEM.

Extract/control	Methanol (mg/mL)				Dichloromethane (mg/mL)				RMP (µg/mL)		DMSO
	10	20	100	200	10	20	100	200	10	20	20%
M. fortuitum ATCC 684	-	10±2.0	13.5±1.5	15±1.0	-	-	-	11±0.0	25±0.0	27±0.0	-
M. smegmatis ATCC 19420	11±0.0	11±1.0	14.5±1.5	17±1.0	-	-	-	-	28±0.0	29.5±0.5	-
M. abscessus	-	9.5±0.5	10.5±1.5	11±1.0	-	-	-	-	20±0.0	20±0.0	-
M. phlei ATCC 19240	12±2.5	13±3.0	13±0.0	14.5±0.0	-	-	-	-	20±0.0	22.5±0.5	-

Diameter of cork borer = 8mm, - = resistance, RMP = Rifampicin.

Table 5. Antimycobacterial screening of C*ordia sebestena* Linn. leaf extracts. Mean diameter of zone of inhibition (mm) ± SEM.

Extract/control	Methanol (mg/mL)				Dichloromethane			(mg/mL)	RMP (µg/mL)		DMSO
	10	20	100	200	10	20	100	200	10	20	20%
M. fortuitum ATCC 684	-	-	18±0.0	20±0.0	-	-	-	-	25±0.0	27±0.0	-
M. smegmatis ATCC 19420	-	9±0.0	16.5±0.5	17.5±1.5	-	-	-	-	28±0.0	29.5±0.5	-
M. abscessus	-	-	-	-	-	-	-	-	20±0.0	20±0.0	-
M. phlei ATCC 19240	-	-	-	-	-	-	-	-	20±0.0	22.5±0.5	-

Diameter of cork borer = 8 mm, - = resistance, ND = Not determined, RMP = Rifampicin.

Table 6. Antimycobacterial screening of C*ordia sebestena* Linn. stem bark extracts. Mean diameter of zone of inhibition (mm) ± SEM.

Extract/control	Methanol (mg/mL)				Dichloromethane (mg/mL)				RMP (µg/mL)		DMSO
	10	20	100	200	10	20	100	200	10	20	20%
M. fortuitum ATCC 684	-	-	20±0.0	23±0.0	-	-	14±0.0	18±0.0	25±0.0	27±0.0	-
M. smegmatis ATCC 19420	12.5±0.5	12.5±0.5	20.5±1.5	20±0.0	-	11±0.0	15±0.0	19±1.0	28±0.0	29.5±0.5	-
M. abscessus	-	-	-	-	-	-	-	-	22±0.0	20±0.0	-
M. phlei ATCC 19240	-	-	10±0.0	13±0.0	-	-	-	-	20±0.0	22.5±0.5	-

Diameter of cork borer = 8 mm, - = resistance, RMP = Rifampicin.

Table 7. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of bioactive extracts in mg/mL.

Extract/control	Ceiba stem bark MeOH		Cordia leaf MeOH		Cordia stem bark MeOH		Rifampicin (µg/mL)	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
M. fortuitum ATCC 684	20	40	200	400	200	400	40	80
M. smegmatis ATCC 19420	20	40	200	600	200	400	40	80
M. abscessus	200	600	R	R	R	R	40	80
M. phlei ATCC 19240	200	600	R	R	100	400	40	80

R= Resistance, ND = not determined.

extract has the highest potency, that is, lower dose of this extract will be required to treat or prevent infections when compared with the amount or dose of other extracts that will treat or prevent infections with the same organisms. The MIC of the methanol extracts of the stem barks of *C. pentandra* and *C. sebestena* and the methanol extract of the leaf of *C. sebestena* for all the tested organisms showed varying concentrations. This gives a clue to effective pharmacologic concentrations of the extracts that will be required to treat infections caused by the

test organisms. Generally, the methanol extracts of the morphological parts of the two different plants had better activity when compared with the dichloromethane extracts. The dichloromethane extracts of some of the plant parts demonstrated no antimicrobial activity at all test concentrations. The antimycobacterial activities of the test plants samples can be attributed to the presence of secondary metabolites detected in the samples. It is noteworthy to make a comparative assessment of the effects of the extracts and the antibiotic used as standard. The control drugs had MIC and MBC values lower than those of the extracts. The antibiotic, though of lower concentrations, is synthetic in nature and expected to show more potency as it is reflected in the zones of inhibition. The effect of the antibiotic as regards the zones of inhibition, however, is not significantly higher than the extracts. The various conditions to which the extracts were subjected during the study could possibly have a direct effect on their antimicrobial activity. The observations above support the use of *C. pentandra* and *Cordia sebestena* in herbal cure remedies, and suggest that these plants may turn out to be a good replacement for the antibiotics in the future with further research focusing on the isolation of the active principle(s) of the plants.

Conclusion

The present study has investigated the *in vitro* antimycobacterial activities of the methanolic and dichloromethane extracts of the stem bark and leaf of the plants *C. pentandra* and *C. sebestena* and compared them with standard antimicrobial agent Rifampicin. The choice of the plants used in the study was based on their reported activities in the treatment of various disease conditions especially diseases resulting from infections of the respiratory tract. The organisms employed in this study were *M. fortuitum* ATCC 684*, M. smegmatis* ATCC 19420*, M. abscessus* and *M. phlei* ATCC 19240*.* All methanol extracts of the stem bark and leaf of the two plants exhibited antimicrobial activities on all the tested organisms, though at varying concentrations due to different concentration and composition of bioactive substances, while the dichloromethane extracts of the plants demonstrated little or no activity.

The inhibitory activities reported in this research have established the antimicrobial properties of *C. pentandra* (L.) Gaertn. and *C. sebestena* Linn**.** The antimicrobial activities of the extracts of the various parts of the plants against the organisms suggest that they can be used in the treatment of infections for which the organisms have been implicated. This justifies the ethnomedicinal use of the plants in the treatment of respiratory infections and associated diseases.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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