Int. J. Pure Appl. Sci. Technol., 19(2) (2013), pp. 76-87

International Journal of Pure and Applied Sciences and Technology

ISSN 2229 - 6107

Available online at ***www.ijopaasat.in***

Research Paper

Anti-Tubercular Compounds from *Spondias Mombin*

Joseph A.O. Olugbuyiro[1,*] and Jones O. Moody[2]

[1] Department of Chemistry, Natural Products Unit, Covenant University, P.M.B. 1023 Ota, Nigeria

[2] Department of Pharmacognosy, University of Ibadan, Ibadan, Nigeria

* Corresponding author, e-mail: (olugbuyiro@yahoo.com)

(Received: 14-9-13; Accepted: 25-10-13)

Abstract: *Spondias mombin is a widely cultivated edible plant used in folkloric medicine for the treatment of severe cough and other respiratory disorders. This study evaluated the anti tubercular property of the stem of S. mombin against Mycobacterium tuberculosis (H37Rv and EJA-2011) using agar proportion assay on Lowenstein-Jensen medium. Four new compounds were isolated from the stem of Spondias mombin and they were identified as mombinrin (**1**), mombincone (**2**), mombinoate (**3**) and mombinol (**4**) respectively. Compound **1** is a coumarin, **2-4** are flavonoids. At 40 µg/mL concentration, the four compounds exhibited significant inhibitions ($p < 0.05$) against M. tuberculosis. At a lower dose of 25 µg/mL, compounds **1** and **3** exhibited significant antimycobacterial inhibitions (96.0 % and 97.6 % respectively; $p < 0.05$) while compounds **2** and **4** showed moderate inhibitions (85.0 % and 88.0 % respectively). The findings show that Spondias mombin accumulates antimycobacterial compounds that may serve as an important potential source for anti-tubercular agents.*

Keywords: *Spondias mombin*, anti-tubercular compounds, antimycobacterial flavonoids, coumarin.

1. Introduction

Spondias mombin Linn. is a widely cultivated economic plant that produces edible floral parts. It grows very easily from stakes to make live fences and enclosures. In W Nigeria the tree serves as shade and the stem is used for making fence. The fruits (Figure 1) are commonly sold in West African markets. Ripe fruits are eaten out-of-hand, or stewed with sugar. The small fruits can be eaten or used in making juices and ice creams. The aromatic fruit of *S. mombin* is rich in vitamins B1 and C [5] [15]. The young leaves are cooked as greens and the green fruits pickled in vinegar and eaten like olives with salt and chilli. A tea of the flowers and leaves is taken to relieve stomachache, biliousness,

urethritis, cystitis, eye and throat inflammations. The plant is used in folkloric medicine for the treatment of various diseases. A decoction of the bark is taken in cases of severe cough with inflammatory symptoms, giving relief through vomiting [5] [22]. The powdered bark is used for treating wounds. The gum is employed as an expectorant and anthelmintic [15]. The plant is reported to have antibacterial, antifungal, and antiviral effects [8] [19] [20]; it also has sedative, antiepileptic, antipsychotic, anti-diarrhoeal, anti-inflammatory, cytotoxicity properties [1] [2] [20]. The leaf of the species has been reported to have an α-amylase inhibitory effect [11]. Although some work has been done on the pharmacological properties of crude extracts of *S. mombin* leaf, there are very few reports on the activities of the stem. We previously reported preliminary antimycobacterial activity of the stem bark [18] [29]. A few studies have been carried out on the phytochemical composition of the leaf of *S. mombin* but there are scanty reports on the constituents of the stem. The phytochemical, proximate, minerals and vitamins A and C compositions of *Spondias mombin* leaves were determined by Igwe *et al* [13]. The plant accumulates phenolic compounds [12] [17]. The isolation of 2-*O*-Caffeoyl-(+)-allohydroxycitric acid and chlorogenic acid butyl ester and a series of 6-alkenyl-salicylic acids has been reported [9] [10]. Moreover, SB-202742, an anacardic acid derivative possessing beta-lactamase inhibitory activity, has been isolated from a hexane extract of the leaf of *Spondias mombin* [7]. In an investigation involving antidiabetic activity, 3β-olean-12-en-3-yl (9*Z*)-hexadec-9-enoate, an α-amylase inhibitor, was isolated from *S. mombin* leaf [11].

Tuberculosis is a major cause of death worldwide. World Health Organization reported [24] that each year there are around nine million new cases of TB, and close to two million deaths. In 2011, there were an estimated 8.7 million new cases of TB (13 % co-infected with HIV) and 1.4 million people died from TB [26]. All countries are affected by this disease. Geographically, the burden of TB is highest in Asia and Africa. India and China together account for almost 40 % of the world's TB cases. The African Region has 24 % of the world's cases, and the highest rates of cases and deaths per capita [26]. The rapid increase in the number of TB cases in the developing countries is alarming [25]. Of great concern is the alarming increase in multidrug-resistant tuberculosis (MDRTB), coupled with the extensive drug-resistant (XDR) strains of *M. tuberculosis*. Worldwide, 3.7 % of new cases and 20 % of previously treated cases were estimated to have MDRTB [24] [26]. All these challenges have made it necessary to identify new antimycobacterial agents with unique structures to combat this global health threatening disease. In the present study we report the isolation of antimycobacterial constituents from the stem bark of *Spondias mombin*.

Figure 1: Fruits of *Spondias mombin* (Bode market in Ibadan, SW Nigeria)

2. Materials and Methods

2.1 Plant Material

The stem bark of *Spondias mombin* L was collected at Badeku in Ibadan, Nigeria in February 2010. The plant was identified by Dr. O. A. Ugbogu of the Forestry Research Institute of Nigeria (FRIN), Ibadan. A voucher specimen (FHI no. 107896) was deposited in the Herbarium of FRIN.

2.2 General Procedure

Vacuum Liquid Chromatography (VLC) was performed on a Si gel (230-400 mesh, Merck; Germany). Gravity column chromatography was performed on a silica gel (70-230 mesh, Merck; Germany). Prep-TLC was carried out on Si gel 60 F_{254} plates (20 x 20 cm, 0.5 mm; Merck, Germany). Analytical TLC on Si gel 60 F_{254} plates (10 x 5 cm, 0.25 mm Merck, Germany). IR spectra were recorded on a Thermo Nicolet 100 FT-IR spectrometer. APCI mass spectra were obtained using either the positive or negative mode. ^{1}H and ^{13}C NMR spectra were run on a Bruker 600 MHz spectrometer, and recorded in $CDCl_3$ using TMS as the internal standard. *Mycobacterium tuberculosis* H37Rv and EJA-2011 strains were provided by the Department of Microbiology, Sacred Heart Hospital, Lantoro Oniko, Abeokuta, Nigeria.

2.3 Extraction and Isolation

The extract was prepared from the dried, pulverized stem bark of *S. mombin* (3.230 kg) by maceration with MeOH. The crude extract was fractionated by VLC on Si gel 230-400 mesh (Merck) with gradient elution using hexane, EtOAc, MeOH and water in the order of increasing polarity to afford eleven fractions. The anti-Mtb active portion was subjected to gravity column chromatography over a silica gel (70-230 mesh) column (110.0 x 3.0 cm) and successively eluted with solvents of increasing polarity starting from *n*-hexane to 100 % MeOH. Twenty-eight fractions (E1-E28) were collected from the column. The anti-Mtb active fractions E8 and E9 were pooled, based on TLC profiles, and then subjected to repeated column chromatography over silica gel to afford compound **1** (11.0 mg). Eluates E16, E17 and E21 from 100 % $CHCl_3$, 100 % $CHCl_3$ and 100 % EtOAc respectively were further purified by a PrepTLC glass plate (Silica gel 60 $F_{254,}$ 20 x 20 cm; 0.5 mm; Merck) and developed in Pet. Ether/EtOAc/MeOH (7:3:0.5), Hexane/EtOAc 7:3 and Pet. Ether/EtOAc (5:5) respectively. Compounds **2**, **3** and **4** were respectively isolated from E16, E17 and E21 fractions.

2.4 Antimycobacterial Activity Test

The antimicrobial susceptibility of *Mycobacterium tuberculosis* was performed by the proportion method on an L-J medium. Inoculums for the proportion method were prepared according to standard procedures [12] [16]. Rifampicin (RIF) was used as the reference drug at a concentration of 40 μg/ml against *M. tuberculosis* H37Rv standard strain. The column fractions and the pure compounds were tested *in vitro* against the clinical isolate (EJA-2011) at concentrations of 40 μg/ml and 25 μg/ml. The prepared bacterial inoculum (0.1 ml) was inoculated on the L-J medium containing the test drug or reference drug or drug-free control and then incubated at 37°C for 28 days. Readings were taken on the 28^{th} and 42^{nd} days of incubation. Each test was done in quadruplicate. Activity in the L-J medium was evaluated by percentage inhibition and calculated by mean reduction in number of colonies on test sample-containing as compared to drug-free controls.

Statistical Analysis

Data was analysed using Student's t-test and analysis of variance (Anova). The significant differences between means were determined at $p < 0.05$.

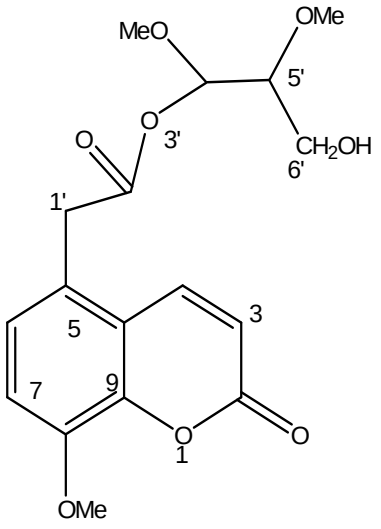
3. Results

The methanol extract of the stem bark of *S. mombin* yielded 161.5 g (5.0 %). Repeated column chromatography of the anti-Mtb VLC fraction (3.829 g) afforded four molecules (**1**-**4**). Compound **1** was isolated as a greenish solid (11.0 mg) and showed characteristic values of R_f 0.89. IR (KBr) υ_{max} 2923 (CH_3), 2852 (CH_2), 1719 (C=O), 1275 (C-O-C) cm^{-1}. ^1H and ^{13}C NMR (Table 1). APCI-MS: *m/z* (rel.int.): 352 $[M]^+$ (37), 338 $[M+H-CH_3]^+$ (92), 237 $[M+H-C_5H_8O_3]^+$ (26), 222 $[M-C_5H_8O_3-CH_3]^+$ (100), 151 $[M- C_5H_8O_3-CH_3-C_3H_4O_2]^+$ (34); (calcd for $C_{17}H_{20}O_8$). Compound **1** was identified as *8-methoxy coumaric acetic acid ester* and named mombinrin.

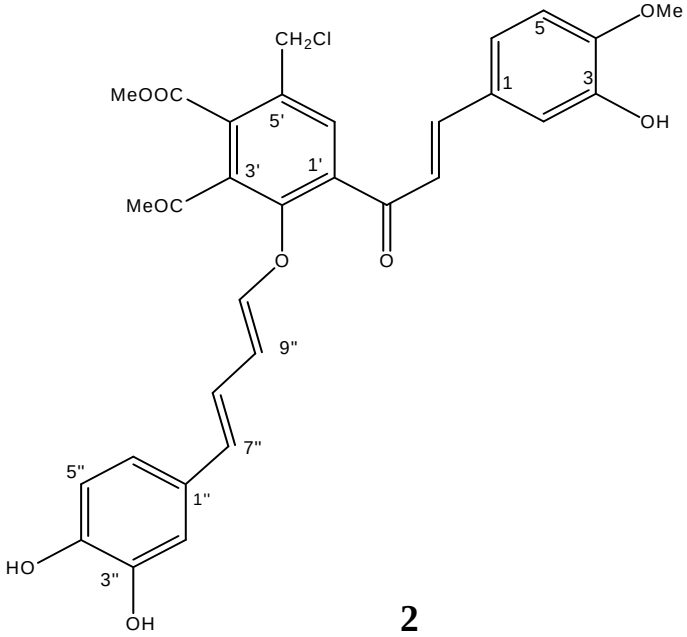
Compound **2** was obtained as a light yellow amorphous solid (4.0 mg) and characterized with R_f 0.75, IR (KBr) υ_{max} 3434 (O-H), 1710 (C=O) cm^{-1}. ^1H and ^{13}C NMR (Table 2). APCI-MS: *m/z* (rel.int.): 578 $[M]^-$ (19), 562 $[M+H-OH]^-$ (28), 543 $[M-Cl]^-$ (34), 527 $[M+H-2OH -H_2O]^-$ (47), 479 $[M+H-2OH-H_2O-CH_2Cl]^-$ (28), 473 $[M+2H-OH-COOCH_3-OCH_3]^-$ (56), 451 (44), 369 $[M+2H-OH-COOCH_3-OCH_3-C_8H_8]^-$ (100), 295 (63), 279 (28), 255 (34), 239 (44), 233 (84), 220 (34), 177 (14). (calcd for $C_{31}H_{27}O_9Cl$). Compound **2** was elucidated as *3'-Acetyl-5'-chloromethyl-2'-(3'', 4''-dihydroxy-phenyl butanoid)-3-hydroxy-4-methoxy-4'- methyl ester chalcone*. It was named mombincone.

Compound **3** was isolated as greenish amorphous solid (2.0 mg) and characterized with R_f 0.72; IR (KBr) υ_{max} 3447 (O-H), 3019 (aromatic), 1690 (C=O), 1216 (C-O-C) cm^{-1}. ^1H and ^{13}C-NMR (Table 3). APCI-MS: *m/z* (rel.int.): 352 $[M]^+$ (41), 338 $[M+H-CH_3]^+$ (21), 311 $[M+H-CH_3 -HCO]^+$ (25), 293 $[M+H-CH_3 -HCO -H_2O]^+$ (43), 279 (21), 277 $[M+H-CH_3 -HCO -2OH]^+$ (38), 193 $[M+H- C_9H_4O_3]^+$ (100), 163 $[M+H- C_{11}H_{10}O_3]^+$ (9); (calcd for $C_{20}H_{16}O_6$). Compound **3** was identified as *7-(3-oxo propionaldehyde)-flavan-3-methyl ester* and named mombinoate.

Compound **4** was isolated yellow amorphous solid (7.0 mg) and characterized as follows: R_f 0.91; IR (KBr) υ_{max} 3447 (O-H), 3018 (aromatic), 1714 (C=O), 1616, 1463 (phenyl) 1216 (C-O-C) cm^{-1}. ^1H and ^{13}C-NMR (Table 4). APCI-MS: *m/z* (rel.int.): 562 $[M+H]^+$ (21), 531 $[M-OCH_3]^+$ 517 $[M-COOH]^+$ (15), 475 (41), 391 (100), 338 (44), 282 (45), 205 (32), 166 (25), 162(16), 149 (69), 116 (21), 113 (48), 107 (34); (calcd for $C_{30}H_{26}O_{11}$). Compound **4** was identified as *3', 4'- dimethoxy-5-(3'', 4''-dihydroxy-phenylbutanoid)-3-hydroxy-6-methoxy-7-methyl flavonoate* and named *mombinol*.



1



2

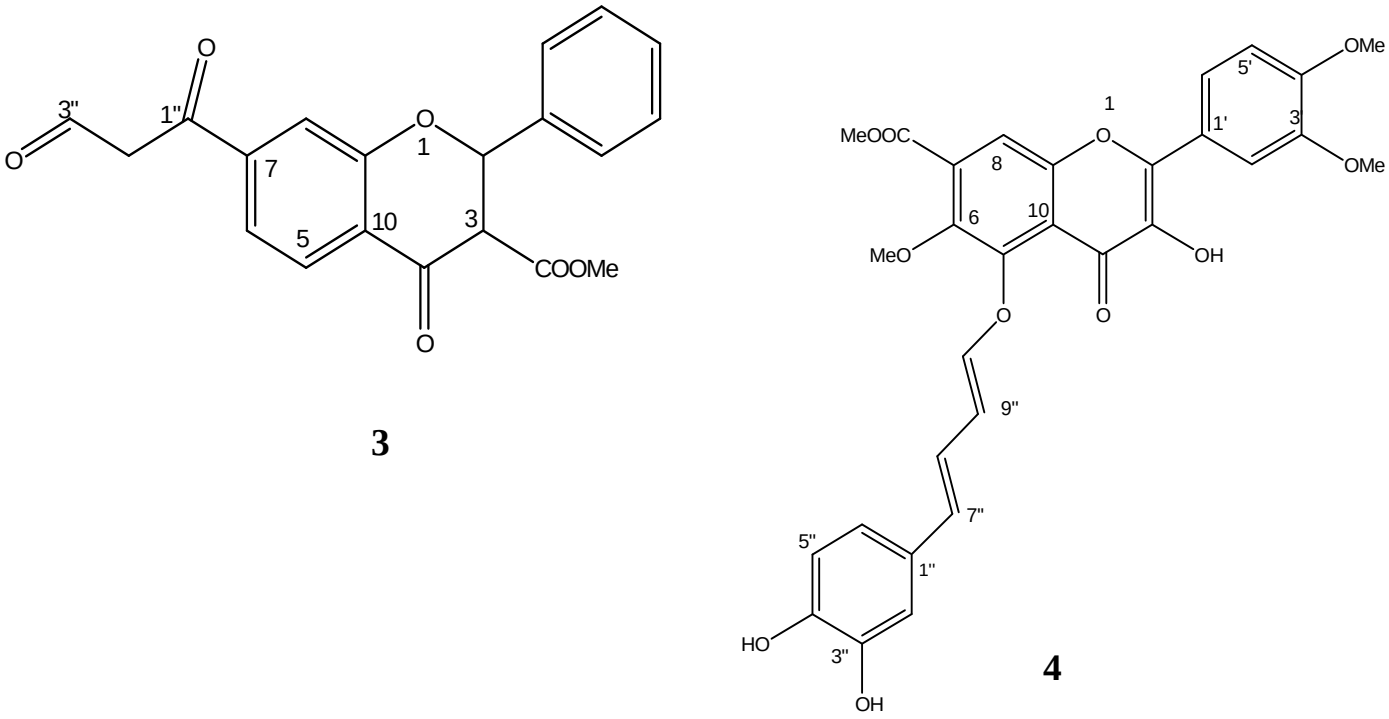


Figure 2: Structures of compounds (**1-4**) from *Spondias mombin*

4. Discussion

The methanol extract of the stem bark of *S. mombin* exhibited a positive response when tested against *Mycobacterium tuberculosis*. Significant antimycobacterial inhibitions ($p < 0.05$) were observed in the activities (Table 5) of the VLC and open column fractions. Fractionation and purification by repeated silica gel column chromatography afforded four compounds (**1-4**) as pure constituents from the stem-bark of *S. mombin*. The IR absorption bands of the compounds (υ_{max} 1719-1690 cm^{-1}) and the ketonic carbon signals observed in their ^{13}C NMR spectra suggested the molecules to be carbonyl containing groups — a coumarin (**1**), a chalcone (**2**) and two flavonoids (**3** and **4**). The elucidation of the four new compounds (Figure 1) were supported by analyses of their MS, ^{1}H and ^{13}C –NMR, HMBC, APT, ^{1}H-^{1}H COSY, and HSQC data in comparison with the literature data [3] [21] [27].

The HMBC spectrum of compound **1** showed long range correlation signals between C-8 (δ_C 157.6) and OCH_3 (δ_H 3.89), C-2^1 (δ_C 171.2) and the germinal protons (δ_H 2.05, 2.02) of the side chain indicating a –CO- CH_2- chain. Homonuclear COSY and ^{2}J-coupling constants revealed the connectivity of the aromatic protons at δ_H 6.86ppm (H-3) to δ_H 7.97 ppm (H-4) and at δ_H 7.95 ppm (H-6) to δ_H 6.86 ppm (H-7). Fragmentation of the compound **1** with coumarin nucleus produced the base peak *m/z* 222 calculated for C_{11} H_{10} $O_{5;}$ peak *m/z* 338 calculated for C_{16} H_{18} O_8 and some major peaks. The ^{1}H- NMR spectrum of **2** exhibited a one-proton *meta*-coupled doublet at δ 7.06 ppm (J = 2.4 Hz) and a one-proton *ortho*-, *meta*-coupled doublet doublet at δ 7.08 ppm (J = 8.4, 2.4 Hz) and a one-proton *ortho*-coupled doublet at δ 6.90 ppm (J= 8.4 Hz) ascribed to aromatic protons H-2^1, H-6^1 and H-5^1 respectively, supporting ABX-type coupling system of ring B. Mass spectral analysis of compound **2** produced peak *m/z* 562 (M+2, *m/z* 564) at the loss of one hydroxyl group and further loss of two molecules of water that led to the formation of a peak at *m/z* 527 ($C_{31}H_{24}ClO_6$) with the attendant M+2 peak (*m/z* 529). This showed the presence of an halogen which was confirmed by the existence of a daughter peak at *m/z* 543 (34%) with the M+1 peak at *m/z* 544 due to the cleavage of chlorine atom from the molecular ion. Furthermore, a loss of CH_2Cl from *m/z* 527 i.e. M+ H-49 (CH_2Cl) gave rise to *m/z* 479 peak. The base peak *m/z* 369, calculated for C_{20} H_{14} ClO_5^+, was a fragment of the molecular ion *m/z* 578 at α-cleavage after the loss of OH, $COOCH_3$ and OCH_3. The

results of the molecular formula analysis showed that the chalcone nucleus contained chlorine element. Fragmentation of compound **3** at α-cleavage produced the base peak m/z 193 calculated for $C_{11}H_{13}O_3$ and peak m/z 163 calculated for $C_9H_7O_3$ (Figure 3). Also, at α- cleavage and σ-bond ruptures compound **4** showed some major peaks (base peak m/z 391, calculated for $C_{20}H_{23}O_8$ and peak m/z 180 calculated for $C_{10}H_{12}O_3$) that confirmed the structural formula.

Agar proportion assay of the anti-tubercular activity of *S. mombin* fractions and pure compounds was conducted at concentrations of 40 µg/mL and 25 µg/mL. At a concentration of 40 µg/mL the four compounds (**1-4**) exhibited significant inhibitions ($p < 0.05$) against *M. tuberculosis*. These inhibitions are comparable to the 99.8 % inhibition exhibited by the reference drug (rifampicin). At a lower concentration (25 µg/mL), compounds **1** (mombinrin) and **3** (mombinoate) still demonstrated significant antimycobacterial activities (96.0 % and 97.6 % inhibition respectively) at 95 % confidence level however, compounds **2** (mombincone) and **4** (mombinol) were less active, with percentage inhibitions of 85.0 % and 88.0 % respectively.

A series of flavonoids, chalcones and chalcone-like compounds have been evaluated [14] for inhibitory activity against *Mycobacterium tuberculosis* H37Rv. Eight compounds from the series exhibited > 90 % inhibition on the growth of the bacteria at a concentration of 12.5 mg/mL. Likewise, there was a report [4] on flavonoid inhibitors acting as novel antimycobacterial agents targeting Rv0636. It was found that butein, isoliquirtigenin, 2, 29, 49-trihydroxychalcone and fisetin inhibit the growth of *Mycobacterium bovis* BCG. Askun *et al.* [28] also investigated the i*n vitro* activity of methanol extracts of plants used as spices against *Mycobacterium tuberculosis* and other bacteria. The study determined the phenolic composition of two thyme species (Lamiaceae), *Origanum minutiflorum* and *Thymbra spicata* and assessed their antimycobacterial activity as well as antibacterial property. The methanol extracts exhibited high level of activity against Mtb (minimum inhibitory concentration MIC 196 µg/ml). In this study, *in vitro* antimycobacterial assay using agar proportion method on L-J medium proved two molecules (**1** and **3)** from *Spondias mombin* stem bark significantly active ($p < 0.05$) against *M. tuberculosis* at a given concentration of 25 µg/mL.

Conclusion

The present study isolates four new molecules, *mombinrin* (**1**), *mombincone* (**2**), *mombinoate* (**3**) and *mombinol* (**4**), from the stem bark of *Spondias mombin*. The four molecules (**1-4**) are reported from a natural source for the first time. The compounds exhibited significant antimycobacterial property that suggests *S. mombin*, a plant that accumulates juicy-fruits, as a potential source for anti-tubercular agents. The folkloric uses of *Spondias mombin* for cough in the West African sub-region are justified by the findings.

Acknowledgements

We thank Prof. J H Van der Westhuizen, Dr. P. Venter and Dr. Anette Allemann of the University of the Free State, South Africa for running the NMR & Mass Spectra of this work. The authors appreciate Mr. Oluwaseun Ejilude, Department of Microbiology, Sacred Heart Hospital, Lantoro Oniko, Abeokuta, for technical support on anti-Mtb test.

Table 1. ^{1}H -and ^{13}C –NMR Spectral Data of **1** ($CDCl_3$, 600 MHz)

C/H No.	^{13}C	PROTON	COSY	HMBC
1	O	…….		
2	159.7	……		
3	115.2	6.86 (1H, d, J = 9.0)	4	
4	131.9	7.97 (1H, d, J = 9.0)	3	
5	128.2	……		
6	129.9	7.95 (1H, d, J = 9.0)	7	
7	114.4	6.86 (1H, d, J = 9.0)	6	
8	157.6	…….		
9	135.4	……		
10	122.8	……..		
1'	37.1	2.05, 2.02 (2H, dd, J =17Hz)		C-2'
2'	171.2	……		
3'	O			
4'	100.1	4.17 (1H, d)		
5'	89.2	4.16 (1H, d)		
6'	60.4	4.04 (2H)		
8-OCH_3	59.4	3.89 (3H, s)		C-8
4'-OCH_3	51.9	3.67 (3H, s)		
5'-OCH_3	59.3	3.63(3H, s)		

Table 2. ^{1}H -and ^{13}C –NMR Spectral Data of **2** ($CDCl_3$, 600 MHz)

C/H No.	^{13}C	PROTON	COSY	HMBC	C/H No.	^{13}C	PROTON	COSY	HMBC
CO	171.2	…..			1"	131.7	……		
α	123.5	7.54 (1H,d, J = 8.4 Hz)		β–C	2"	126.5	7.30 (1H,d, J = 2.4 Hz)		
β	141.1	7.55 (1H,d, J = 8.4 Hz)			3"	139.3	……..		
1	129.9	…..			4"	138.4	…….		
2	116.5	7.06 (1H,d, J =2.4 Hz)			5"	123.9	7.13 (1H, dd, J = 6.0 Hz)		
3	147.1	……			6"	124.1	7.14 (1H, d, J = 6.0 , 2.4 Hz)		
4	147.7	…….			7"	130.9	6.61 (1H, d, J = 7.8 Hz)	8"	
5	115.9	6.90 (1H,d, J =8.4 Hz)	6	C-4	8"	124.8	7.08 (1H, m)	7" , 9"	
6	119.1	7.08 (1H,dd, J =8.4, 2.4 Hz)	5	C-2	9"	124.5	5.83 (1H, m)	8"	
1'	114.1	……			10"	142.9	6.51 (1H, d, J = 7.8 Hz)		C-2'
2'	165.8	…….			4-OCH_3	65.1	4.12 (3H, s)		
3'	128.1	…….			4'a-OCH_3	64.7	4.10 (3H, s)		
3'a-CO	173.8	…….			3-OH		5.01 (1H, s)		
4'	135.8	……..			3''-OH		4.98 (1H, s)		
4'a-CO	173.4	……..			4"-OH		4.97 (1H, s)		
5'	129.1	…….			3'b-CH_3	22.7	2.60 (3H, s)		C-3'
6'	130.2	7.35 (1H, s)			5'-CH_2Cl	44.5	4.92 (3H, s)		

Table 3. ^{1}H -and ^{13}C –NMR Spectral Data of **3** ($CDCl_3$, 600 MHz)

C/H No.	^{13}C	PROTON	COSY	HMBC
2	87.5	6.28 (1H,d, J = 10.2 Hz)		C-9
3	65.2	3.91 (1H, d,)		C-3a
4	182.5	…		
5	125.3	7.61 (IH, d, J = 8.4 Hz)		
6	113.4	7.54 (1H, d, J =8.4 Hz)		
7	143.7	…		
8	103.2	7.36 (1H, s, t)		C-9
9	158.6	…		
10	107.4	…		
1'	142.6	…		
2'	128.0	7.13 (IH, dd, J = 8.4, 2.4 Hz)	3'	
3'	129.7	7.08 (1H, dd, J = 8.4, 2.4 Hz)	2'	
4'	129.0	6.92 (1H, dd, J = 8.4)		
5'	129.9	7.07 (IH, dd, J = 8.4, 2.4 Hz)	6'	
6'	128.2	7.12 (1H, dd, J =8.4, 2.4 Hz)	5'	
1"	192.9	…		
2"	55.9	3.93, 3.92 (2H, d)	3''	C-3"
3"	203.8	9.77 (1H, t)	2"	
3a	171.2			
3a-OCH_3	56.4	3.95 (3H, S)		

Table 4. ^{1}H -and ^{13}C –NMR Spectral Data of **4** ($CDCl_3$, 600 MHz)

C/H No.	^{13}C	PROTON	COSY	HMBC	C/H No.	^{13}C	PROTON	COSY	HMBC
1	O	…..			6"	124.1	7.07 (1H, dd, J = 6.0, J = 2.4 Hz)		
2	135.9	….			7"	130.9	6.90 (1H, dd, J = 6.0 Hz)	8"	
3	128.8	……			8"	124.8	7.08 (1H, dd; J = 6.0, J = 2.4 Hz)	7", 9"	
4	173.4	….			9"	124.5	5.85 (1H, m)	8"	
5	151.8	….			10"	139.3	6.51 (1H, d, J = 18)		C-5
6	143.1	….			6-OCH_3	68.2	2.87 (3H, s)		
7	135.2	…..			7b-OCH_3	60.4	4.06 (3H, s)		C-7a
7a	167.8	….			3-OH		5.08 (1H, s)		C-2
8	123.5	6.99 (1H, s)		C-9	3'-OMe	64.7	3.78 (3H, s)		
9	152.1	….			4'-OMe	63.1	3.80 (3H, s)		
10	114.1	….			3"-OH		7.36 (1H, s)		
1'	130.9	….			4"-OH		7.35 (1H, s)		
2'	116.1	7.54 (1H, d, J = 2.4 Hz)		C-3'					
3'	147.6	….							
4'	147.1	….							
5'	115.9	6.60 (1H, d, J = 8.4 Hz)	6'						
6'	119.1	7.53 (1H, d, J = 8.4, 2.4 Hz)	5'						
1"	131.2	….							
2"	126.5	7.30 (1H, d, J= 2.4 Hz)							
3"	142.9	….							
4"	138.5	….							
5"	123.9	7.13 (1H, d, J = 6.0 Hz)		C-6"					

Table 5. *In vitro* antimycobacterial activity of *Spondias mombin* extracts using agar Proportion method (Lowenstein Jesen [L-J] medium)

Fraction/Isolate	**Nature**	**Microorganism**	**L-J proportion method**	
			% Inhibition	
			Fraction/Isolate	
			40µg/ml	25µg/ml
VL2	VLC fraction	*M. tuberculosis* EJA-2011	97.8	93.7
VL3	VLC fraction	*M. tuberculosis* EJA-2011	76.2	65.0
E8-9	column fraction	*M. tuberculosis* EJA-2011	98.2	96.5
E16	column fraction	*M. tuberculosis* EJA-2011	92.0	87.4
1	isolate	*M. tuberculosis* EJA-2011	98.2	96.0
2	isolate	*M. tuberculosis* EJA-2011	93.1	85.0
3	isolate	*M. tuberculosis* EJA-2011	99.7	97.6
4	isolate	*M. tuberculosis* EJA-2011	92.1	88.0
Control drug				
RIF		*M. tuberculosis* H37Rv	99.8	N.T

RIF- Rifampicin

References

[1] P.I. Akubue, G.C. Mittal and C.N. Aguwa, Preliminary pharmacological study of some Nigerian medicinal plants, *J. Ethnopharmacol*, 8(1983), 53-63.

[2] A.O. Ayoka, R.O. Akomolafe, E.O. Iwalewa, M.A. Akanmu and O.E. Ukponmwan, Sedative, antiepileptic and antipsychotic effects of *Spondias mombin* L. (Anacardiacaea) in mice and rats, *J Ethnopharmacol*, 103(2006), 166-175.

[3] E. Breitmaier and W. Voelter, Carbon-13 NMR Spectroscopy: High-Resolution Methods and Applications in Organic Chemistry and Biochemistry (3rd ed.), VCH Verlagsgesellschaft, Weinbein, 1989.

[4] A.K. Brown, A. Papaemmanouil, V. Bhowruth, A. Bhatt, L.G. Dover and G.S. Besra, Flavonoid inhibitors as novel antimycobacterial agents targeting Rv0636: A putative dehydratase enzyme involved in *Mycobacterium tuberculosis* fatty acid synthase II, *Microbiology*, 153(2007), 3314-3322.

[5] H.M. Burkill, The Useful Plants of West Tropical Africa (Vol. 1) (2nd ed.), Royal Botanic Gardens, Kew, 1995.

[6] R.E. Chaisson and N.E. Martinson, Tuberculosis in Africa: Combating an HIV-driven crisis, *N. Engl. J Med*, 358(2008), 1089-1092.

[7] N.J. Coates, M.L. Gilpin, M.N. Gwynn, D.E. Lewis, P.H. Milner, S.R. Spear and J.W. Tyler, SB-202742 a novel beta-lactamase inhibitor isolated from *Spondias mombin*, *J Nat Prod*, 57(1994), 654-657.

[8] F.J. Corthout, L.A. Pieters, M.V. Claeys, D.A. Berghe and A.J. Vlietinck, Antiviral: Ellagitannins from *Spondias mombin*, *Phytochemistry*, 30(1991), 1129-1130.

[9] J. Corthout, L. Pieters, M. Claeys, S. Geerts, D.V. Berghe and A. Vlietinck, Antibacterial and molluscicidal phenolic acids from *Spondias mombin*, *Planta Med*, 60(1994), 460-463.

[10] J. Corthout, L. Pieters, M. Claeys, D.V. Berghe and A.J. Vlietinck, Antiviral caffeoyl: Esters from *Spondias mombin*, *Phytochemistry*, 31(1992), 1979-1981.

[11] A. Fred-Jaiyesimi, K. Abo and R. Wilkins, α-Amylase inhibitory effect of 3β-olean-12-en-3-yl (9*Z*)-hexadec-9-enoate isolated from *Spondias mombin* leaf, *Food Chemistry*, 16(2009), 285-288.

[12] R. Gupta, B. Thakur, P. Singh, H.B. Singh, V.D. Sharma, V.M. Katoch and S.V.S. Chauhan, Anti-tuberculosis activity of selected medicinal plants against multi-drug resistant *Mycobacterium tuberculosis* isolates, *Indian J Med Res*, 131(2010), 809-813.

[13] C.U. Igwe, G.O.C. Onyeze, V.A. Onwuliri, C.G. Osuagwu and A.O. Ojiako, Evaluation of the chemical compositions of the leaf of *Spondias Mombin* linn from *Nigeria*, *Australian Journal of Basic and Applied Sciences*, 4(5) (2010), 706-710.

[14] Y.M. Lin, Y. Zhou, M.T. Flavin, L.M. Zhou, W. Nie and F.C. Chen, Chalcones and flavonoids as anti-tuberculosis agents, *Bioorg. Med. Chem.*, 10(2002), 2795-2802.

[15] J. Morton, Yellow Mombin, In: J.F. Morton, Fruits of Warm Climates, Miami, FL, 1987.

[16] National Committee for Clinical Laboratory Standards (NCCLS), Susceptibility testing of mycobacteria, nocardia and other aerobic actinomycetes: Approved standard, Document M24-A, Wayne, PA, 2003.

[17] P.C. Njoku and M.I. Akumefula, Phytochemical and nutrient evaluation of *Spondias mombin* leaves, *Pakistan Journal of Nutrition*, 6(6) (2007), 613-615.

[18] J.A.O. Olugbuyiro, J.O. Moody and M.T. Hamann, AntiMtb activity of triterpenoid-rich fractions from *Spondias mombin* L., *Afri J Biotechnol*, 8(2009), 1807-1809.

[19] K.F. Rodrigues and M. Hesse, Antimicrobial activities of secondary metabolites produced by endophytic fungi from *Spondias mombin*, *J Basic Microbiol*, 40(2000), 261-267.

[20] A.R.A. Silva, S.M. Morais, M.M.M. Marques, D.M. Lima, S.C.C. Santos, R.R. Almeida, I.G.P. Vieira and M.I.F. Guedes, Antiviral activities of extracts and phenolic components of two *Spondias* species against dengue virus, *J Venomous Anim Toxins Trop Dis*, 17(2011), 406-413.

[21] R.M. Silverstein, G.C. Bassler and T.C. Morrill, Spectroscopic Identification of Organic Compounds (5th ed.), John Wiley & Sons, New York, 1991.

[22] L.N.D. Taylor, Ubos (*Spondias mombin*), URL: http://rainforest-database.com/plants/ubos.html, Accessed on 23.09.2012, (2006).

[23] L.F. Villegas, T.D. Fernadz, H. Maldonado, R. Torres, A. Zavaleta, A.J. Vaisberg and G.B. Hammond, Evaluation of wounds healing of selected plants from Peru, *J Ethnopharmacol*, 55(1997), 193-200.

[24] World Health Organization, The global plan to stop TB 2011–2015, WHO Report, Geneva 27, Switzerland, 2010.

[25] World Health Organization, Global tuberculosis control, WHO/HTM/TB/2011.16, Geneva 27, Switzerland, 2011.

[26] World Health Organization, Global tuberculosis report, WHO/HTM/TB/2012.6, Geneva 27, Switzerland, 2012.

[27] J. Buckingham (Ed), Dictionary of Natural Products on CD-ROM, The Chapman & Hall/CRC Chemical Database, 2011.

[28] T. Askun, G. Tumen, F. Satil and M. Ates, *In vitro* activity of methanol extracts of plants used as spices against *Mycobacterium tuberculosis* and other bacteria, *Food Chemistry*, 116(2009), 289-294.

[29] J.A.O. Olugbuyiro, J.O. Moody, M.T. Hamann, Phytosterols from *Spondias mombin* Linn. with antimycobacterial activities, *African Journal of Biomedical Research*, 16 (1) (2013),182-186.