ISSN 2278- 4136

ZDB-Number: 2668735-5

IC Journal No: 8192

Volume 1 Issue 3

Online Available at www.phytojournal.com

Journal of Pharmacognosy and Phytochemistry

Anti-TB Activity of *Sterculia setigera* Del., Leaves (Sterculiaceae)

Ibrahim T. Babalola[1,3], Esther A. Adelakun[2*], Yuehong Wang [3] Francis O. Shode[4]

1. Dept. of Chemistry, Faculty of Science, Yobe State University, PMB 1144 Damaturu, Nigeria.
2. Dept. of Chemistry, Faculty of Science, University of Jos, Jos, Nigeria. [E-mail: adelakun@unijos.edu.ng]
3. Institute for Tuberculosis Research, College of Pharmacy, University of Illinois at Chicago, 833S, Wood Street Chicago, IL 60612, United States.
4. Department of Medicinal Chemistry, Faculty of Sciences, University of Zululand, South Africa.

The use of herbs (medicinal plants) for the treatment of diseases is as old as the history of mankind. *Sterculia setiger*a is an example of such plant endowed in nature to serve the needs of man. Folkloric claims on antibacterial activity of *S. setigera* were investigated *in-vitro* on a micro-scale using the Alamar Blue Assay. Three of four successive solvent extractions of the plant leaves extracts inhibited the growth of a virulent strain of *Mycobacterium tuberculosis*, H37Rv (ATCC27294) in the concentrations tested (1-128 µg/ml). The minimum inhibitory concentration (MIC) determined for the hexane, dichloromethane and ethyl acetate extracts were 84 µg/ml, 62 µg/ml and 128 µg/ml respectively. The combined active extracts were subjected to an extensive bioactivity-guided fractionation using a combination of normal phase (NP VLC) and reverse phase (RP MPLC) chromatography. Purification of active fractions led to pure sub-fractions with good activity (MICs 15.13 µg/ml-31.43 µg/ml). The cytotoxicity effect of the most active fractions was determined against African green monkey kidney cells (Vero cells) and human hepatocellular carcinoma (HepG2) cell lines. The fractions were non-toxic to Vero and HepG2 cells. Two compounds have been isolated from these active sub-fractions and structural elucidation is being carried out. The results show that *S. setigera* leaves are active against *M. tuberculosis*, and the anti-TB activity of ethnomedicinal used *S. setigera* leave has been confirmed by *in vitro* tests for the first time. This current report further suggests the presence of potential anti-mycobacterial agent(s) or anti-TB drug leads in *S. setigera* leaves.

Keyword: Folkloric Medicine, *Sterculia setigera*, Natural Products, Anti-TB Activity, *Mycobacterium tuberculosis,* Drug Discovery

1. Introduction

According to the World Health Organization (WHO), *Mycobacterium tuberculosis* (MTB) has infected one -third of the world's population and resulted in the death of approximately 1.3-1.75 million patients in 2007 alone. MTB infection has been increasing in parallel with a rising rate of human immunodeficiency virus (HIV). At the same time, multidrug-resistance (MDR-TB) and extensive drug resistance TB (XDR-TB)) is a growing problem of serious concern in many countries[1]. In Nigeria the situation is also alarming. The country has been ranked as the 5th among the 22 high TB burden countries. Information on TB cases has reported an increase from 31,264 in 2002 to 90,307 in 2008. Nigeria now has 1,000,000 new TB cases and is globally rated 3rd after Rwanda and Malawi. TB burden in

Nigeria is compounded by the high HIV/AIDS prevalence and poverty[2-3].

Herbal medicines and other alternative therapies are increasingly used for TB treatment, especially against drug resistant TB and multidrug-resistant tuberculosis. It is widely accepted that natural products are a proven template for the development of new scaffolds of drugs[4].

The panacea to the global health threat emerging from TB, MDR-TB and XDR-TB could be unveiled through screening of medicinal plants marked by the indigenous people to have therapeutic activity in the treatment of respiratory diseases. In recent years, a significant number of natural product derived agents have been discovered by employing screening approaches involving cellular or biochemical targets in their assay design[7]. Anti-mycobacterial active compounds have been found among many skeleton types, mainly from plants, but also from other organisms such as fungi and marine organisms.

The plethora of structures reported to have anti - TB activity are summarized in three reviews, one focusing on plant terpenoids[8], and the others on providing a comprehensive summary by structural types and including other organisms as well[9-11]. In Nigeria, large numbers of medicinal plants belonging to different genera are used for the treatment of various diseases including suppurative lung diseases.

As part of our contribution to the global search for new anti-TB compounds, we unearthed an age-long secret of TB treatment from a family (traditional Fulani healers) in a remote village called Narabi, in Bauchi State, Nigeria. The plant in question is known to many tropical West African communities, but the information on anti-TB potency of the plant leaves has not been reported.

Sterculia species are well represented in West Africa. Among them are two species mostly used in folk medicine - *S. setigera,* Del and *S. tragacantha* Lindl. *Sterculia setigera*, Del (synonyms- *S. tomentose*, Guill&Perr), is a savanna tree wide spread in tropical Africa, sometimes up to 40 ft high and 5 ft girth. It is commonly known as kukkuki in Hausa; Bo'poli in Fulani; Shadarat addamn by Shuwa and Sugubo in Kanuri. It is locally referred to as Wol and Mapp in Senegal and Karaya gum plant in English. It is characterized by pale purplish bark with thin scales which peel off to expose yellowish patches; slash crimson, exuding a gummy sap. Traditionally, the plant has been used for the treatment of boils, whitlow, inflammations, chicken–pox, measles, dysentery, syphilis, epilepsy, jaundices, malaria and leprosy[12-14]. The anti -bacterial and anti-fungal activities of dried bark, dried fruit and the root of *S. setigera* against common microbes species such as *Bacillus Subtilis*, *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Aspergillus niger* and *Candida albicans* have been reported[13],[15]. The anti-viral activity of *S. setigera* against three human and three animal viruses (Poliovirus (type 1), astrovirus, human herpes simplex virus (type 1), equine herpes simplex virus, bovine parvovirus and canine parvovirus have also been reported[16]. Investigation on *S. setigera* over the years regarding its therapeutic activity has focused on the seed, gum, root and the bark[17-19]. Decoctions of the powdered dried leaves of the plant are commonly used for the treatment of TB, and in HIV/AIDS patients with chronic cough with blood stains (Personal information from Alh. Salihu Muhammad, 80yr old Fulani traditional healer of Narabi village, Bauchi state, Nigeria (deceased)).

In order to provide scientific evidence for this ethnomedical claim, we initiated bioassay screening of *S. setigera* leaf extracts against virulent *M. tuberculosis*, $H_{37}Rv$ (ATCC27294), using the Alamar Blue Assay.

This paper presents a preliminary report on the anti-TB activity of *S. setigera* using bio-assay guided fractionation of the extracts of the leaves and fractions thereof.

2. Materials and Methods

2.1. Plant Material

The leaves of *S. setigera* used for this study were collected in September, 2008, around rocky features behind Narabi village, Sabongari, Bauchi

state, Nigeria. The plant material was authenticated by Dr. I. Abdulkareem of the Federal College of Forestry, Jos, Plateau State, Nigeria.

2.2. Preparation of Extracts

The freshly collected plant materials were kept on the laboratory bench, air dried and pulverized into coarse powder.

A micro-scale extraction was carried out by packing 6.0 g of the plant material into a 10ml sterile pipette; defatting with hexane and successively extracting with Dichloromethane (DCM), ethylacetate (EtoAc) and methanol (MeOH). Pasteur pipettes were used to transfer solvent into the pipette and pipette bulb to force the solvent through the packed plant material. The extracts were collected in 50m ml flasks, evaporated using a Buchi rotary evaporator at 37 ^{o}C.

The concentrated extracts were completely air-dried under air stream at room temperature and weighed. The process afforded soluble fractions of HEX (160 mg), DCM (140 mg), EtOAc (20 mg) and MeOH (520 mg) with yields of 2.7%, 2.3%, 0.33% and 8.7% respectively. Samples of 2.0 mg of the each of the successive extracts were transferred into sterile 1-ml vials for anti-TB bioassay. Following the promising results obtained from the pilot study (micro-scale extraction) of *S. setigera*, 0.7 kg of the pulverized leaves was packed into a 2000 ml separating funnel. The separating funnel was connected to a tank (2.5 L bottle) with Teflon tubing and rubber stopper.

The tank is suspended above the funnel with the help of clamps to make a percolation set-up. The material was first defatted with hexane (2.5Lx2), and the solvent removed in vacuo using Buchi-RotoVap at 37 °C.

The defatted material was then successively and exhaustively extracted with dichloromethane followed by ethylacetate.

The extracts were combined and completely evaporated and dried in a dessicator to afford 41.2 g of the crude extract (yield =5.8%). 40.0 gm of the crude extract was loaded dry by adsorption on silica gel and fractionated by vacuum liquid chromatography (VLC, 800 g, 60 M, 0.04-0.063 mm/230-400 mesh silica gel), eluting with a gradient of hexane-ethyl acetate-methanol (5% stepwise increase). A total of forty fractions were collected and concentrated in vacuo. 2.0 mg of each of the fractions were transferred into sterile microtube for anti-Tb assay. Based on the normal phase TLC profile of the fractions and the results of anti-Tb activity, similar fractions were pooled together to afford six main fractions (A, B, C, D, E & F).

Fraction E (1000 mg) was further fractionated by repeated NP VLC eluting with gradient solvent system-hexane-ethyl acetate-methanol (10% stepwise increase). Twenty three fractions collected were spotted and developed on normal phase TLC, like fractions were pooled together to obtain five main fractions-E1.1(3.3 mg), E1.2 (2 mg), E1.3 (682 mg), E1.4 (35.8 mg) & E1.5 (113 mg). 2.0 mg of each of these fractions were transferred into sterile microtube for anti-Tb assay.

2.3. Purification of Fraction E1.3

A sample (300 mg) of fraction E1.3 was purified by reverse phase medium pressure liquid chromatography (RP MPLC) using FMI pump at 1.6 ml/min (Fluid Metering Pump, Model QG 50,New York, USA).The sample (300 mg) was dissolved in 2.5 ml 98% methanol and injected into 3ml sample loop using a 3ml syringe) and loaded onto an equilibrated RP C18 pre-packed column (Grobe B (310-25), 40-63 µM, product of Merck, Darmstadt Germany). The sample was eluted with gradient solvent system of methanol-water, 50%, 6o%, 70%, 80%, 85%, 90 and 98%). Based on the TLC profile, fractions collected at the various gradient steps were combined and designated fractions (E1.3.1, E1.3.2, E1.3.3, E1.3.4&5, E1.3.6, E1.3.7).

2.4. Further Purification of E1.3.4&5

Based on the observations of the chromatogram for fractions obtained at 80-85% methanol-water (280-370), fractions were recombined and purified further on a smaller pre-packed RP C18 column (GrobeB (240-10),40-63 µM, product of

Merck, Darmstadt Germany); eluted with a smaller gradient of methanol –water (75%, 77%, 80%, 85%, 90% and 98%). A total of 306 fractions were collected.
The fractions were spotted and developed on NP TLC using chloroform-methanol-TFA (80:19:1). Like fractions were pooled to obtain eighteen sub-fractions. 1mg samples of each fraction were transferred in to micro-tube for anti-TB assay.

2.5. Bacterial Strains for anti-TB biological assays

For the preparation of the inoculum, a virulent strain of *M. tuberculosis*, H37Rv (ATCC27294) bacteria was grown in 100 ml of Middlebrook 7H9 Broth (Difco, Detroit, MI), supplemented with 0.2% (v/v) glycerol (Sigma Chemical Co., St Louis, MO), 10% (v/v) OADC (oleic acid, albumin, dextrose, catalase; Difco), an 0.05% (v/v) Tween 80 (Sigma), also referred to as 7H9GC-T80.

2.6. Microplate Alamar Blue Assay (MABA)

Anti-TB susceptibility testing of extracts and isolates was determined in the fluorometric Microplate Alamar Blue Assay (MABA) as described previously [20], [21]. The extracts MICs against MTB H37RV (ATCC27294) were assessed by the MABA using rifampin and isoniazid as positive controls.
Sample stocks were prepared in DMSO at concentration of 12.8 mM, and the final test concentrations range from 128 µM to 0.5 µM. Two fold dilutions of compounds were prepared in Middlebrook 7H12 medium (7H9 Broth containing 0.1% w/v casitone, 5.6 µg/mL palmitic acid, 5 mg/mL bovine serum albumin, 4 mg/mL catalase, filter-sterilized) in a volume of 100 µL in 96-well Microplates (BD Optilux, 96-well Microplates, black/clear flat bottom). MTB cultures (100 µL inoculum of 2 x 10^5 cfu/ mL) were added, yielding a final testing volume of 200 µL. The plates were incubated at 37 ^{0}C. On the seventh day of incubation 12.5 µL of 20% Tween 80, and 20 µL of Alamar Blue (Invitrogen BioSource^{TM}) were added to the wells. After incubation at 37 ^{0}C for 16-24 hrs, fluorescence of the wells were measured (ex 530, em 590 nm). The MIC was determined as the lowest concentration effecting a reduction in fluorescence of ≥ 90% relative to the mean of replicate bacteria-only controls.

2.7. Cytotoxicity Assay

Evaluation of the cytotoxicity activity of isolates in Vero cells (African green monkey kidney cells) was performed as described previously[8] using the Cell Titer 96 aqueous radioactive cell proliferation assay (Promega Corp., Madison, WI). The IC_{50} was defined as the reciprocal dilution resulting in 50% inhibition of the Vero cells. The cytotoxicity was determined by exposing the Vero cells to different concentrations of the samples. Stock solutions of the samples were prepared at 12.8 mg/mL.
The positive control rifampin (RMP) at 100 mg/ML in DMSO. Geometric six fold dilutions were performed in growth medium MEM (Gibco, Grand Island New York), containing 10% fetal bovine serum (Hyclone, Logan, UT). Final DMSO concentrations did not exceed 1% v/v. Drug dilutions were distributed in duplicate in 96-well tissue culture plates (Becton Dickinson labware, Lincoln Park, NJ) at a volume of 50 µL per well. An equal volume containing 5x10^5 Vero cells (CCL-81; American Type Culture Collection, Rockville, MD) was added to each well and incubated at 37 ^{0}C in an atmosphere of 5% CO_2 in air. After 72hours, cell viability was measured using the CellTiter 96 aqueous non-radioactive cell proliferation assay according to the manufacturer's instructions. Absorbance at 490nm was read in a victor II reader (Perkin Elmer Life Sciences Inc., Boston, MA). The IC_{50} was determined using a curve-fitting program. The results are presented in Table 3.

Table 1: Result of Anti-TB Activity of Micro -scale Extraction of *Sterculia setigera* leaves

Code	Solvent	Percentage Inhibition at Tested Concentrations					MIC (µg/ml)
		8 µg/ml	16 µg/ml	32 µg/ml	64 µg/ml	128 µg/ml	
SSE-1	HEX	35	56	48	83	93	84.34
SSE-2	DCM	75	78	75	91	90	62.51
SSE-3	ETOAC	45	57	69	77	90	128
SSE-4	MEOH	35	11	12	33	39	>128
RMP	DMSO	96	98	99	100	100	0.06

Strain: H37RV, ; Stock Conc: 12.8 mg/ml,; Test Conc: 128-8 ug/ml; RMP: Rifampin

3. Results and Discussion

The result of anti-TB assay of the extracts of *S. setigera* (leaves) shows a remarkable activity against virulent strain of *Mycobacterium tuberculosis* ($H_{37}R_V$) in three out of the four crude extracts tested. The hexane, dichloromethane and ethyl acetate fractions inhibited the growth of the bacterium significantly, comparable to the inhibition observed using the reference drug (rifampin). The activity of the plant extracts is concentration dependent with 62 µg/ml and 128µg/ml concentrations exhibiting greater inhibition (Table 1). The potency of the plant extract is more remarkably consistent in the dichloromethane fraction (DCM), exhibiting MIC of 62.51 µg/ml and the hexane fraction (MIC 84.34 µg/ml) while the EtOAc fraction (128 µg/ml) showed less inhibitory activity than the DCM fraction. The lipophilic extractives exhibited greater activity than the Ethylacetate and Methanol (Table 1). Further purification of the active subfraction E (E1.3.4&5) led to simple fractions with high activity (MICs 31.43 µg/ml, 29.68 µg/ml and 15.13 µg/ml (Table 3). The *in vitro* cytotoxicity values (IC_{50}) of 102.4 µg/ml and 99.35 µg/ml for subfractions (E1.3.4&5.7 and (E1.3.4&5.8) respectively as well as 81.08 µg/ml and 81.69 µg/ml indicates non cytotoxic and probably a good indicator for the candidature of this plant extract as possible source of drug candidate.

Table 2: Results of Anti-TB Assay of sub- fractions (E) of *Sterculia setigera.*

Code	Percentage Inhibition at Tested Concentrations							MIC (µg/ml)
	2 µg/ml	4 µg/ml	8 µg/ml	16 µg/ml	32 µg/ml	64 µg/ml	128 µg/ml	
E1.1	0	0	12	42	45	49	66	>128
E1.2	31	53	68	79	93	94	93	28.65
E1.3	5	23	28	35	68	98	99	55.52
E1.4	15	52	54	68	79	94	93	54.84
E1.5	0	0	0	2	32	92	94	63.01
RMP	48	83	97	100	100	101	100	0.05

Strain : H37Rv; Stock conc.:12.8 mg/ml; Test conc. :128 -8 µg/ml

Table3: Results of Anti-TB Assay of sub-fractions E1.3.4&5 of *Sterculia setigera*

Fraction Code	Percentage Inhibition at Tested Concentrations					MIC µg/ml	IC_{50} µg/ml Vero	IC_{50} µg/ml HepG2
	8 µg/ml	16 µg/ml	32 µg/ml	64 µg/ml	128 µg/ml			
E1.3.4&5.1	-6	-6	-6	6	54	>128	NT	NT
E1.3.4&5.2	-21	0	7	8	61	>128	NT	NT
E1.3.4&5.3	-14	-15	25	65	55	>128	NT	NT
E1.3.4&5.4	-1	-7	37	45	72	>128	>128	>128
E1.3.4&5.5	-1	2	87	99	99	38.99	101.2	>128
E1.3.4&5.6	-3	9	71	100	100	53.21	87.21	44.1
E1.3.4&5.7	20	98	100	100	100	15.13	102.4	81.08
E1.3.4&5.8	15	32	100	100	100	29.68	99.35	81.69
E1.3.4&5.9	-11	-22	22	91	100	63.66	108.2	80.71

E1.3.4&5.10	-5	-16	15	29	100	118.27	35.46	24.56
E1.3.4&5.11	-29	8	27	33	45	>128	NT	NT
E1.3.4&5.12	-50	13	27	29	71	>128	NT	NT
E1.3.4&5.13	-24	14	-3	18	15	>128	NT	NT
E1.3.4&5.14	-25	23	-15	10	17	>128	NT	NT
E1.3.4&5.15	-16	18	1	36	24	>128	NT	NT
E1.3.4&5.16	-3	60	91	93	93	31.43	25.44	30.22
E1.3.4&5.17	10	41	70	86	90	128.00	31.75	28.44
E1.3.4&5.18	-10	32	28	44	45	>128	NT	NT
RMP	95	99	99	99	99	0.05	185.3	97.85

Strain: H37Rv; Stock conc.:12.8 mg/ml; Test conc.:128-8 µg/ml ; NT- Not tet tested.

Preliminary examination of the isolates suggests long chain fatty acid with a glycoside unit. Previously reported work has implicated fatty acids as the antibacterial principles in *Diplotaxis harra* and *Erucaria microcarpa*[23], also in the fruit of *Kigelia africana.* Interestingly, pharmacological activity reported in some members of the family- Sterculiaceae were attributed to long chain fatty acids[25].

4. Conclusion

The plant family- Sterculiaceae has been the subject of investigation for many years considering the economic importance and medicinal application of the plant in folkloric treatment of different ailments[16, 20, 22]. However, the information on the therapeutic effect of the leaves of this plant in the treatment of tuberculosis seems to be an age- long secret known to few members of a Nigerian Fulani family. The focus of all the available literatures was on the seed, root and the stem bark.

The stem bark of the plant was recently reported to have insignificant inhibitory activity against clinical isolates of *M. tuberculosis* and a strain of *Mycobacterium bovis*[22].

The findings in this study have provided scientific support for the ethnomedical anti – TB activity of extracts of the leave of *Sterculia setigera*. We are currently studying the isolates for full characterization; we are equally looking at antiplasmodial as well as anti-inflammatory activity of the plant leave extract.

5. Acknowledgements

The authors are grateful to the Authority of Yobe State University (Nigeria) in conjunction with Education Trust Fund, ETF for the Research Training Fellowship (Babalola) and Institute for Tuberculosis Research (UIC, USA) for the support. The professional assistance on this work by Prof. James McAlpine (adjunct professor at Dept. of Med. Chemistry, UIC), technical assistance received from Dr Shao-Nong Chen and Dr. Tanja of Botanical Research Center, Faculty of Pharmacy, UIC Chicago is hereby acknowledged.

6. Reference

1. WHO. Multidrug and extensive drug resistant TB (M/XDR.TB): 2010 global report on surveillance and response. WHO/HTM/TB/2010.3 WHO Library Cataloguing-in-Publication Data; Geneva, Switzerland, 2010.
2. Espinal MA, Azlo A, Simonsen L, Boulahbal F, Kim SJ, Renoro A *et al*.. Global trends in resistance to anti-tuberculosis drugs. WHO International Union against Tuberculosis and Lung Diseases. Working Group on anti-Tuberculosis Drug Resistance Surveillance. Engl J Med 2001; 344:1294-1303.
3. Nigeria: TB-The Nation Records 1 Million New Cases-(Excerpt from Daily Champion, 25th March 2010). http://www.allafrican.com. 20 April, 2010.
4. Cragg GM, Newman DJ, Suader M. Natural Products in drugs discovery and development. Journal of Natural Products 1997; 60:52-60.
5. Newman DJ, Cragg GM, Snader KM. The influence of natural products upon drug discovery. Natural Products Reports, 2000, 17:215-234.
6. Newman DJ, Cragg GM, Snader KM. Natural products as source of new drugs over the period 1981-2002. Journal of Natural Products 2000; 66:1022-1037.
7. Shu YZ. Recent natural products based drug development: A pharmaceutical industry perspective. Journal of natural prod 1998; 61:1053-1071.
8. Cantrell CL, Rajab MS, Franzblau SG, Fronezek FR, Fischer NH.). Antimycobacterial ergosterol-5, 8-endoperoxide from *Ajuga remota*. Planta Medica 1998; 65:732-734.

9. Copp BR. Antimycobacterial natural products. Natural Products Reports 20, 535-557. Plants used for treatment of respiratory diseases, African Journal of Biotechnology 2003; 7(11):1630-1636.
10. Okunade AL, Elvin-Lewis MP, Lewis WH. Natural antimycobacterial metabolites: current status. Phytochemistry 2004; 65:1017-1032.
11. Pauli GF, Ryan JC, Taichi I, Yuehong W, Sanghyun C, Franzblau SG *et al*. New perspectives on natural products in TB drug research. Life science 2005; 78: 485-494.
12. Dalziel JM. The Useful Plants of West Tropical Africa. Crown Agents for Oversea Government and Administration 1937; 109.
13. Almagboul AZ, Bashiru AK, Karim A, Farouk A, Salihu M, Farouk A *et al*. Antimicrobial Activity of Certain Sudanese Plants Used in Folklorik Medicine. Fitoterapia 1988; 59(5):393-396.
14. Igoli JO, Ogaji OG, Tor-Anyiin TA, Igoli NP. Medicinal Practices among the Igede people of Nigeria Part II, African Journal of Complementary and Alternative Medicine 2005; 2.
15. Almagboul AZ, Bashiru AK, Farouk A, Salih AK. Antimicrobial Activity of Certain Sudanese Plants Used in Folklorik Medicine: Screening for Antibacterial Activity (1V). Fitoterapia 1988; 56(6):331-337.
16. Kudi AC, Myint SH. Antiviral Activity of Some Nigeria Medicinal Plant Extracts. Journal of Ethnopharmacology 1999; 68:289-294.
17. Collins L, Franzblau SG. Microplate alamar blue assay versus BACTEC 460 system for high-throughput screening of compounds against *Mycobaterium tuberculosis* and *Mycobacterium avium*. Antimicr. Agents Chemother 1997; 41:1004-1009.
18. Falzari K, Zhu Z, Pan D, Liu H, Hongmanee P, Franzblau SG. *In vitro* and *In Vivo* Activities of Macrolide Derivatives against *Mycobacterium tuberculosis*, Microbial Agents and Chemoteraphy, 2005; 49(4):1447-1454.
19. Cantrell CL, Rajab MS, Franzblau SG, Fronezek FR, Fischer NH. Antimycobacterial ergosterol-5, 8-endoperoxide from *Ajuga remota*. Planta Medica 1998; 65:732-734.
20. Atawodi SE, Bulus T, Ibrahim S, Ameh DA, Nok AJ, Mamman M *et al*. *In vitro* Trypanocidal effect of methanolic extract of some Nigerian savannah plants. African Journal of Biotechnology 2003; 2(9):317-321.
21. Atawodi SE, Ameh DA, Ibrahim S, Andrew JN, Nzelibe HC, Onyike EO *et al*. Indigenous knowledge system for treatment of trypanosomiasis in Kaduna state of Nigeria. Journal of Ethnopharmacology 2002; 79:279-282.
22. Mann A, Amupitan JO, Oyewale AO, Okogun JI, Ibrahim K, Oladosu P *et al*. Evaluation of *in vitro* antimycobacterial activity of Nigerian Plants used for treatment of respiratory diseases. African Journal of Biotechnology 2008; 7(11):1630-1636.
23. Hashem FA, Saleh MM. Antimicrobial components of some Crucifererae plants (*Diiplotaxis harra* Forsk and *Erucaria microcarpa* Bioss) Phytoteraphy Research. 1999; 13:329-332.
24. Grace OM, Light ME, Lindsey KL, Mulholland DA, Van Staden J, Jager AK. Antibacterial activity and isolation of active compounds from fruit of the traditional African medicinal tree *Kigelia africana*. South African Journal of Botany 2002; 68:220-222.
25. Reid KA, Jager AK, Light ME, Mulholland DA, Van Staden J. Phytochemical and pharmacological screening of Sterculiaceae species and isolation of anti-bacterial compounds. Journal of Ethnopharmacology 2005; 97:285-291.