Scholars Academic Journal of Biosciences (SAJB) **ISSN 2321-6883 (Online)**
Sch. Acad. J. Biosci., 2015; 3(4):386-391 **ISSN 2347-9515 (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com **DOI :** 10.36347/sajb.2015.v03i04.012

Research Article

C*lerodendrum capitatum*(Willd) leave extracts inhibits bacteria, fungi and *Mycobacterium bovis*

Momoh, H[1,2*], Habila, J. D[1]., Ayo, R. G[1], Ndukwe, G.I[1]
[1]Department of Chemistry, Ahmadu Bello University Zaria-Nigeria
[2]Department of Chemistry, Federal University DutseJigawa-Nigeria

***Corresponding author**
Momoh H
Email: momohhajara@yahoo.com

Abstract: Folkloric medicinal application of *Clerodendrum capitatum* in the treatment of tuberculosis was investigated. Phytochemical analysis revealed the presence of carbohydrates, cardiac glycosides, steroids, triterpenes, alkaloids, tannins and saponins. Hexane (HE), dichloromethane (DCM), ethyl acetate (EA) and methanol (ME) extracts of *C.capitatum* leaves were evaluated for antibacterial and antifungal activities, against nine pathogenic bacteria and three fungi; *Shigella dysenteriae, Salmonella typhi, Corynebacterium ulcerans, Klebsiella pneumonia, Staphylococcus aureus, Methicillin resistant Staphillococus aureus, Proteus mirabilis,* and *Streptococcus pneumoniae, Candida tropicalis, Candida krusei* and *Candida albicans*, using the agar-in-well diffusion method. Determination of zone of inhibition (ZI) showed inhibition ranging from 17-26 mm (HE), 23-26 mm (DCM), 26-29 mm (EA) and 20-23 mm (ME) against the entire test organisms except *Corynebacterium ulcerans*. The results of the minimum inhibitory concentration (MIC) showed that EA fraction inhibited the growth of all test organisms at a low concentration of 3.25 mg/mL except *S. aureus* and *C. albicans* which were inhibited at 7.5 mg/mL. Higher MIC values were observed for HE (7.5-15 mg/mL), DCM and ME fraction all showed MIC at 7.5 mg/mL. The microorganisms were completely killed at a higher concentration; EA (MBC/MFC; 7.5-15 mg/mL), DCM (MBC/MFC; 15 mg/mL), ME (MBC/MFC; 15-30 mg/mL) and HE (MBC/MFC; 30 mg/mL). Antituberculosis evaluation reveals that the DCM extract had the highest activity with MIC of 0.675 mg/mL against *Mycobacterium bovis*. The results clearly showed that the plant had potential that can be explored in the search for anti-TB drug.
Keywords: *Clerodendron capitatum*; Antituberculosis; Mycobacterium bovis; Antibactrial; Aantifungal

INTRODUCTION

Tuberculosis (TB) remains a devastating global public health problem, especially in developing countries like Africa [3]. Globally, it has been observed that more than one-third of the world's population (about 2 billion) is infected with the bacterium that causes TB [11]. Approximately 9 million people are diagnosed with the disease of which 2 million people die annually [5, 16]. Furthermore, the disease condition is made worse by the emergence of drug-resistant strains which render the treatment even more difficult. TB treatment is always characterized by long regime of treatment and multiple drug combination. These have highlighted the need for a drug discovery pipeline to ensure the availability of new chemical entities with improved mechanisms of anti-TB action and shorter duration of treatment [20].

In Nigeria, Clerodendrum capitatum has been wide implicated in traditional medicinal application in the treatment of tuberculosis, fever, obesity, diabetes mellitus, diarrhoea, asthma, hypertension and erectile dysfunction [18]. In Sudan, the roots of this plant are used traditionally in the management of male erectile dysfunction [9]. The genus *Clerodendrum* has been reported to demonstrate versatile biological activities by other researchers, such as; antitumorgenic [8], hypoglycemic, hypolipidemic [4], hepatoprotective activity against CCl_4-induced liver injury in rats [6], anti-inflammation [2], radical-scavenging activity [1, 21], antidiarrhoeal [15], antinociceptive, and antipyretic effects [10]. In the current investigation, we report our findings on the biological evaluation of the methanol, ethyl acetate, dichloromethane and hexane extracts of leaves of *Clerodendrum capitatum* against pathogenic bacteria, fungi and *mycobacterium bovis* in order to detect new sources of antimicrobial and antitubercular agents.

EXPERIMENTAL

Plant material

The plant material was collected fresh from Zaria, Nigeria in September, 2013. Taxonomical identification was done at the Herbarium of the Biological Sciences Department, A.B.U, Zaria, Nigeria and its voucher specimen with number 900688

deposited there. The plant was air-dried under shade, segregated and pulverized by mechanical pounding using wooden mortar and pestle. The pulverized plant material was stored away from moisture until needed.

Extraction of plant materials

The pulverized leaves of *Clerodendrum capitatum* (500 g) was carefully weighed and macerated with 95% methanol for one weeks. The extract was decanted, filtered and labeled. The process was repeated three times for exhaustive extraction. The three sets of extracts were combined on confirmation by TLC. The combined methanol extract was partitioned with hexane, dichloromethane and ethylacetate. The extracts were concentrated in vacuum at 40˚C using rotary evaporator and later subjected to air drying to give dried crude extracts.

Phytochemical screening

The hexane, dichloromethane, ethyl acetate and the methanol extracts of the plant was subjected to phytochemical screening using standard techniques [7]. The metabolites tested for included, carbohydrates, tannins, saponnis, flavonoids, anthraquinones, cardiac glycosides, steroids, terpenes and alkaloids.

Antimicrobial studies

The antimicrobial activities of the HE, DCM, EA and ME extracts and standard drugs (Ciprofloxacin, Sparfloxacin and Fluoconazole) were determined using microbial strains and fungi obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria (ABUTH).The test microorganisms used are *Shigella dysenteriae, Salmonella typhi, Corynebacterium ulcerans, Klebsiella pneumoniae, Staphylococcus aureus, Methicillin resistant staphyllococcus aureus* (MRSA), *Proteus mirabilis, Streptococcus pneumoniae, Candida tropicalis, Candida krusei* and *Candida albicans*. The well diffusion method of [14], was used to determine the antibacterial activity of the test extracts. Pure cultures of the bacterial organisms were inoculated on to Mueller Hinton Agar (MERCK) and incubated for 24 h at 38 ^{o}C. About 5 discrete colonies were aseptically transferred using sterile wire loops into tubes containing sterile normal saline (0.85% NaCl) and were adjusted to a turbidity of 0.5 MacFarland Standard. The suspensions were then inoculated on the surface of sterile Mueller – Hinton Agar plates using sterile cotton swabs. A sterile 6 mm diameter Cork borer was used to make holes (wells) into the set of inoculated Mueller-Hinton Agar. The wells were filled with different concentration of the test extracts. The plates were incubated for 24h at 38 ^{o}C. All the tests were performed in triplicate and the antibacterial activities were determined as mean diameters of inhibitory zone (mm) produced by the test extracts.

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentrations (MIC) were determined for the extracts using micro broth dilution method in accordance with [22]. Serial dilution of the least concentration of the extracts that showed activity were prepared using test tubes containing 9 ml of double strength nutrient broth (OXOID). The test tubes were inoculated with the suspension of the standardized inocula and incubated at 38 ^{o}C for 18 h. Minimum inhibition Concentrations (MIC) were recorded as the lowest concentrations of the compounds showing no visible growth (turbidity) in the broth.

Minimum Bactericidal Concentration (MBC/MFC)

The minimum bactericidal and minimum fungicidal concentration were determined by aseptically inoculating aliquots of culture, from the minimum inhibition concentration (MIC) tubes that showed no growth, on sterile nutrient Agar (OXOID) plates and incubated at 38^{o}C for bacteria and 34^{o}C for fungi for 48 h. The MBC/MFCs were recorded as the lowest concentration of extracts showing no bacterial growth at all.

Antituberculosis studies

Microbroth dilution method in Sterile 96 microwell plate was employed for the determination of antimycobacterial activity of the extracts as described by [13]. About 100 mg of each extract was transferred into sterile bottles, dissolved with 0.5 ml dimethyl sulphoxide (DMSO) and 0.5 ml distill water. The extracts were further diluted (1:10) in 7H9 Middle brook broth to give 10 mg/ml concentration. Into each of the 96 microwell plate was transferred 50 µl of sterile 7H9 broth starting from well 2 to 12. To each of the first wells was added 100µl of 10% DMSO in sterile media (prepared by dispensing 0.1 ml of DMSO into 9.9 ml of 7H9 broth as control), 100 µl of 25 µg/ml solution of rifampicin (standard) and 100 µl of each plant extract. Using a multi-channel pipette, 50µl was carefully removed from well 1 and added to well 2, mixed thoroughly by pipetting up and down four times, and the process continued to well 11 from which 50 µl was withdrawn and discarded.

Inoculation

The 5-7 day old culture of BCG monitored on UV spectrophotometer at 650 nm (OD 0.2-0.3) was diluted 1/1000 by adding 50 µl cell culture to 50 ml 7H9/ADC medium, where 50 µL of diluted culture was inoculated to all wells of the plate. The plates were incubated at 30 ^{o}C for 7 days and after incubation stained with tetrazolium dye for growth/inhibition of organisms. The column number of the row at which no apparent growth was seen was recorded as activity.

RESULTS AND DISCUSSION

Phytochemical screening

Phytochemical screening (Table 1) of the crude methanol, ethyl acetate, dichloromethane and

hexane extracts revealed the presence of carbohydrates, cardiac glycosides, alkaloids, tannin, flavonoids, Saponins, steroids and triterpenes. These phytochemicals could be responsible for the antimicrobial and antituberculosis activities exhibited by the extract and hence justify the ethnomedicinal uses of *C. capitatum.*

Antimicrobial screening

The antimicrobial activity of the extract showed that all the extracts exhibited moderate to good antibacterial and antifungal activity against all the pathogens tested *except Corynebacterium ulcerans, Klebsiella pneumonia and candida tropicalis* (Table 2).The ethyl acetate extract exhibited the highest zone of inhibition (29 mm) against *Shigella dysenteriae and Salmonella typhi.*The highest inhibition of fungal growth (28 mm) was recorded against *C. krusei*. Whereas hexane extract exhibited the lowest zone of inhibition (17 mm) against *streptococcus pneumonia* and (18 mm) against *C. albicans and C. krusei* (Table 3).The ethyl acetate extracts exhibited minimum inhibitory concentration (MIC) at 3.25mg/ml against all the pathogenic organism except *S. aureus* and *C. albican* which had MIC at 7.5mg/ml (Table 4.) The MBC showed that the extract was bactericidal at 15 mg/ml against all of the test microorganism (Table 5.)

The anti-TB evaluation

The Antituberculosis activity of the extract showed that the dichloromethane, ethyl acetate and methanol extract were sensitive against *Mycobacterium* bovis, but hexane extract was not. The dichloromethane extract showed the highest activity with minimum inhibitory concentration of 0.625mg/ml , ethyl acetate and methanol extracts howed equal activity with MIC at 2.5mg/ml (Table 6).

Table 1: Phytochemical screening of the extracts of the leaves of *Clerodendron capitatum*

Metabolites	HE	DCM	EA	ME
Carbohydrate	-	+	+	+
Cardiac glycoside	+	+	+	+
Tannins	-	-	+	+
Saponins	-	-	-	+
Flavonoids	-	-	+	+
Anthraquinones	-	-	-	-
Steroids	+	+	+	+
Triterpenes	+	+	+	+
Glycosides	+	+	+	+
Alkaloids	-	+	+	+

Key: + = present, - = absent, HE = hexane extract, DCM = dichloromethane extracts, EA = Ethyl acetate extracts, ME = Methanol extracts

Table 2: Zones of Inhibition (mm) of extractsand standard drugs

Test organism	HE	DCM	EA	ME	Ciprofloxacin	Fluconazole	Sparfloxacin
MRSA	20	23	27	23	35	-	32
S. aureus	18	24	26	22	37	-	41
S. peumoniae	17	25	28	22	38	-	37
S.dysenteriae	19	26	29	23	39	-	40
S. typhi	18	24	29	21	41	-	32
K. pneumonia	0	0	0	0	40	-	37
E.coli	18	23	27	20	32	-	35
P. mirabilis	19	24	28	20	Nil	-	36
C. albicans	18	23	26	21	Nil	36	-
C. krusei	18	24	28	21	Nil	34	-
Candida tropicalis	Nil	Nil	Nil	Nil	Nil	35	-

Key: HE = Hexane extract, DCM= Dichloromethane extracts, EA = Ethyl acetate extracts, ME = Methanol extracts, Ciprofloxacin = 5mg/ml, Sparfloxacin = 5mg/ml, Fluconazole = 5mg/ml

Table 4: Minimum Inhibitory Concentration (MIC)

	Concentration (mg/mL)			
Test organism	**HE**	**DCM**	**EA**	**ME**
MRSA	7.5	7.5	3.25	7.5
S. aureus	15	7.5	7.5	7.5
S. peumoniae	15	7.5	3.25	7.5
S. dysenteriae	15	7.5	3.25	7.5
S. typhi	15	7.5	3.25	7.5
K. pneumonia	15	7.5	3.25	7.5
E.coli	15	7.5	3.25	7.5
P. mirabilis	15	7.5	3.25	7.5
C. albicans	15	7.5	7.5	7.5
C. krusei	15	7.5	3.25	7.5

Key: HE = Hexane extract, DCM= Dichloromethane extracts, EA = Ethyl acetate extracts, ME = Methanol extracts

Table 5. Minimum Bactericidal /Fungicidal Concentration (MBC/MFC)

Test organism	**HE**	**DCM**	**EA**	**ME**
MRSA	30	15	15	30
S. aureus	30	15	15	30
S. peumoniae	30	15	15	30
S. dysenteriae	30	15	7.5	15
S. typhi	30	15	15	30
K. pneumonia	30	15	15	30
E.coli	30	15	15	30
P. mirabilis	30	15	15	30
C. albicans	30	15	15	30
C. krusei	30	15	15	30

Table 6: Minimum Inhibitory Concentration (MIC) of the extracts against *Mycobacterium bovis*

Conc. (mg/ml)	**HE**	**DCM**	**EA**	**ME**	**Rf**
5	-	+	+	+	+
2.5	-	+	+*	+*	+
1.25	-	+	-	-	+
0.625	-	+*	-	-	+
0.3125	-	-	-	-	+
0.156	-	-	-	-	+

Key:- = no inhibition; **+** = inhibition; **+*** = MIC; **Rf** = rifampicin

Phytochemical screening (Table 1) of the crude methanol, ethyl acetate, dichloromethane and hexane extracts revealed the presence of cardiac glycosides, steroids and triterpenes in all the four extracts, alkaloids and carbohydrate were absent only in the hexane extract, tannins and flavonoids were present in the ethyl acetate and methanol extracts. Saponins were present only in the methanol extract, while anthraquinones were absent in all the four extracts. The use of *Clerodendron capitatum* in the treatment of tuberculosis and other ailment seem to be justified, by the presence of the different phytochemicals in the plant. It has been reported that the major activity of medicinal plants against ailments, is a function of the amount of phytochemicals it can produce [12]. Flavonoids have been reported to be effective antibacterial agent, against a wide array of microorganism. Due to their ability to complex with extracellular, soluble proteins and bacterial cell walls leading to membrane disruption [19]. Antibacterial and antifungal results (Table 2 - 5) showed that all the extracts exhibited moderate to good antibacterial and antifungal activities. Zone of inhibition (ZI) determination shows inhibition ranging from 17-26 mm (HE), 23-26 mm (DCM), 26-29 mm (EA) and 20-23 mm (ME) against the entire test organism except *Corynebacterium ulcerans*. As compared to the standard drugs used as positive control (ciprofloxacin, sparfloxacin; 32 - 42 mm, Fluconazole; 34 – 36 mm). The EA was found to be the most active with ZI of 29 mm against *S. dysentriae* and *S. typhi*, ZI of 28 mm was recorded against *C. krusei*. The results of the minimum inhibitory concentration (MIC, Table 4) showed that EA extract inhibited the growth of all test organisms at

a low concentration of 3.25 mg/ml except *S. aureus* and *C. albicans* which were inhibited at 7.5 mg/ml. Higher MIC values were observed for HE (7.5-15 mg/ml), DCM and ME extract showed MIC at 7.5 mg/ml. The microorganisms were not only inhibited but completely killed at a higher concentration; EA (MBC/MFC; 7.5-15 mg/ml), DCM (MBC/MFC; 15 mg/ml), ME (MBC/MFC; 15-30 mg/ml) and HE (MBC/MFC; 30 mg/ml). The broad spectrum exhibited by the extracts from this plant *C. capitatum* as shown in the antimicrobial results lend credence to the traditional uses of the plant in folk medicine. The anti-TB evaluation shows that the plant extracts inhibits the growth of *Mycobacterium bovis*, DCM extract showed the highest activity with MIC of 0.625 mg/ml. The EA and ME extracts inhibited the growth of *Mycobacterium bovis* at MIC of 2.5 mg/mL, no activity was recorded with HE fraction. The use of *C. capitatum*in the treatment of TB and other diseases in Northern Nigeria seem to be justified and offer possibilities that can explore in the search for new drugs.

CONCLUSION

The results of this investigation support the claims by other researchers that medicinal plants inhibit *Mycobacterium bovis* [20]. Therefore, offer endless possibility in the search for lead candidates from plants that could serve as antibacterial, antifungal and anti-TB drug(s). *Clerodendron capitatum* is indeed a useful breakthrough in the search for alternative natural medicine for the treatment of tuberculosis and various diseases.

Acknowledgement

Authors are thankful to the Department of Chemistry Ahmadu Bello University Zaria, Nigeria and National Institute for Pharmaceutical Research and Development, Nigeria (NIPRD), for providing laboratory facilities.

REFERENCES

1. Chae S, Kang KA, Kim JS, Hyun JW, Kang SS; Trichotomoside :Anew antioxidative phenyl propanoid glycoside from *Clerodendron trichotomum*. Chemistry and Biodiversity, 2006 3; (1): 41–48.
2. Choi H, Whang WK, Kim HJ; Studies on the anti-inflammatory effects of *Clerodendron trichotomum* Thunberg Leaves. Archives of Pharmacology Research, 2004; 27 (2): 189–193.
3. Claude K, Paul W, Moses J, Olwa O ;The anti-mycobacterial activity of *Lantana camara* a plant traditionally used to treat symptoms of tuberculosis in South-western Uganda.African Health Science, 2009; 9(1): 40–45.
4. Devi R, Sharma DK; Hypolipidemic effect of different extracts of *Clerodendron colebrookianum* Walp in normal and high-fat diet fed rats.; Journal of Ethnopharmacology, 2004; 90 (1): 63–68.
5. Dye C, Scheele S, Dolin P, Pathenia V, Raviglione MC; Global Burden of TB: estimated incidence, prevalence and mortality by country. JAMA,1999; 282: 677–686.
6. Gopal N, Sengottuvelu S; Hepatoprotective activity of *Clerodendrum inerme* against CCL induced hepatic injury in rats. Fitoterapia, 2008; 79 (1): 24–26.
7. Harborne JB; Phytochemical methods: A guide to modern techniques of plant analysis. 2nd edition, *Chapmann and Hall*, London,1973; 279 pp.
8. Liu S, Zhu H, Zhang S , Zhang X , Yu Q , Xuan L; Abietanedi terpenoids from Clerodendrum bungei. .Journal of Natural Products, 2008; 71(5) : 755–759.
9. Mahmoud A, Khidir MO, Khalifa M A, El Ahmadi AB, Musnad HA, Mohamed E I; Sudan: Country Report to the Fao International Technical Conference on Plant Genetic Resources .1995;67.
10. Narayanan N, Thirugnanasambantham P, Viswanathan S, Vijayasekaran V, Sukumar E; Antinociceptive, anti-inflammatory and antipyretic effects of ethanol extract of *Clerodendronserratum* roots in experimental animals. Journal of Ethnopharmacology, 1999; 65 (3): 237–241.
11. NavinTR, Mc Nab SJ; Crawford J T; The continued threat to tuberculosis**.** EmergInfec Dis, 2002 ; 8 : 1187.
12. Ojiako EN; Phytochemical analysis and antimicrobials screening Of *Moringa Oleifera* Leaves Extract. International Journal of Engineering and science, 2014; 3 : 32-35.
13. Oladosu PO,Isu NR, Ibrahim K, Orishade AT, Oladepo D , Lovett L; Antituberculosis activity of bioactive compounds from fruits extracts of Acacia nilotica. Journal of microbiology Research, 2013; 3 : 247-254.
14. Preeti G, Uday V, Singh T; Phytochemical screening and antimicrobial activity of some medicinal plants against oral flora. Asian Pac. Journal of Health Sci. , 2014 ;1 : 255-263.
15. Rani S, Ahamed N, Rajaram S, Saluja R, Thenmozhi S, Murugesan T;, Anti-diarrhoeal evaluation of *Clerodendrum phlomidis* Linn. leaf extract in rats. Journal of Ethnopharmacoly, 1999; 68 (1–3): 315–319.
16. SanjayMJ; Natural Products: An important source for Anti-tubercular Drugs. CRISP, 2004; 5: 1
17. Sasidharan S., Chen Y, Saravanan D,Sundram K M, Yoga L L; Extraction, isolation and characterization of bioactive compounds from plantsextracts. African Journal of Traditional, Complementary and Alternative Medicine.2010; 8(1) :1-10.
18. Siddig IA, Abdelwahab HM, Osama YM , Mahjoub O, Manal M E T, Syam M, Mohamed I.N, Mohd R S, and Khalid M. A. Erectogenic Effects of *Clerodendroncapitatum*: Involvement of Phosphodiesterase Type-5 Inhibition, Evidence

Based Complementary and Alternative Medicine, 2012; 2012 : 6 Pages

19. Stern J L, Iiagerman AE, Steinberg PD, Masion Phorotannin PK;. protein interactions, Journal Chem Ecol, 1996; 22 :1877–99.
20. Valerie FDD, Susana R, Gabriela T, Patrick VTF, LauveYT, Nole T, Paul HAZ, Margarida CNF ;.Antimycobacterial activity of selected medicinal plants extracts from Cameroon. Int. J. Biol. Chem Sci, 2014; 8 (1): 273-288.
21. Vidya SM, Krishna V, Manjunatha BK, Mankani KL, Ahmed M, Singh SDJ; Evaluation of hepatoprotective activity of *Clerodendrumserratum*. Indian Journal of Experimental biology, 2007 ; 45 (6): 538–542.
22. Vollekova A , Kostalova D, Sochorova R; Isoquinoline alkaloids from *Mahoniaaquifolium* stem bark are active against Melssezia species. Folia Microbiology, 2001; 46 : 107-111.