Sahel Journal of Life Sciences FUDMA (SAJOLS)
December 2023 Vol. 1(1): 229-236
ISSN: 3027-0456 (Print)
ISSN: xxxx-xxxx (Online)
DOI: *https://doi.org/10.33003/sajols-2023-0101-025*

Research Article

Phytochemical Screening and Some Antibacterial Activities of *Clerodendrum capitatum* Leaves Extract

***Abubakar Rimi Dahiru, Aminu Bello Riji and Musbahu Buhari**
Department of Applied Chemistry, Faculty of Physical Sciences, Federal University Dutsin-Ma, Nigeria
***Corresponding Author:** adahirurimi@fudutsinma.edu.ng

Received: 3rd December, 2023 **Accepted**: 13th December, 2023 **Published**: 31st December, 2023

ABSTRACT

Plant were used for medicinal purposes since ancient times for treatment of diverse ailments. *Clerodendrum capitatum* has been used in the treatment of tuberculosis, fever, obesity, diabetes mellitus, diarrhoea, asthma, etc. In this study, *C. capitatum* leaves were extracted using standard procedures and then subjected to phytochemical screening, thin layer chromatography (TLC), and column chromatography. The single spot isolated compound (I1) obtained from the column was subjected to antimicrobial analysis, bioautography, Fourie Transform Infrared (FT-IR) analysis. The phytochemical screening indicates the presence of secondary metabolite like alkaloid and terpenoid. The n-hexane extract TLC show 8 spots with different Retention factors (R_f) value, then after column was done, compound with R_F value 0.35 was isolated from the column chromatography. Antimicrobial test result showed that the Isolate was active against *Salmonella typhi, Bacillus megaterium, Staphylococcus epidermidis, Klebsiella spp, Escherichia coli, Pseudomonas aeruginosa, Proteus spp, Shigella spp, Bacillus subtilis* and *Staphylococcus aureus* (with zones of inhibition range 7mm-17mm), highest activity was recorded against *Staphylococcus epidermidis*, and least activity against *Bacillus megaterium* both at concentration of 100mg/L. The bioautography also show zone of inhibition with white coloured visibility. The FT-IR analysis revealed that the isolates contain ketone functional group (RCOR) group. The presence of the secondary metabolite like alkaloids and terpenes in the crude extract and Ketone functional group in the isolate suggest the reasons for the antibacterial activity of the isolated compound.

Keywords: Medicinal Plants; Alternative Medicine; Plant Extracts; FTIR; Bacteria

Citation: Dahiru, A. R., Riji, A. B. and Buhari, M. (2023). Phytochemical Screening and Some Antibacterial Activities of *Clerodendrum capitatum* Leaves Extract. *Sahel Journal of Life Sciences FUDMA*, 1(1): 229-236. DOI: *https://doi.org/10.33003/sajols-2023-0101-025*

INTRODUCTION

Up to 80% of the world's population, according to estimates from the World Health Organization (WHO), receives some primary healthcare from traditional medical systems (Yan *et al.,* 2018). It is commonly recognized in African ethnomedicines that traditional healers employ a wide range of plants to treat parasitic illnesses, such as malaria, and that many of the herbal therapies that traditional healers prescribe are seen as beneficial by their patients (Yan *et al.,* 2018). Hundreds to thousands of linked chemical compounds with various biological and therapeutic effects can be found in natural goods. Utilizing natural materials derived from plants, animals, or microorganisms to create novel therapeutics is becoming more and more popular (Epole *et al., 2020*). Secondary metabolites, such as flavanoids, iridoids, and phenolic chemicals, are abundant in the genus *Clerodendrum* (Mostapha *et al.,* 2013). Over the past few decades, the boundaries of the genus *Clerodendrum* have undergone significant alteration. The genus is polyphyletic in its traditional circumscription, as demonstrated by molecular markers. The genus *Clerodendrum* has split apart in

order to preserve its monophyly, with certain genera—like *Volkameria* and *Rotheca* (Pierre*et al.,* 2023). Numerous biological activity, including anticancer, hypoglycemic, anti-inflammatory, and anti-diarrheal properties, have been described for species of the *Clerodendrum* genus (Habila *et al.,* 2018). The Verbenaceae family, which includes *Clerodendrum capitatum,* is made up of small trees, shrubs, and plants with a tropical and subtropical range. *C. capitatum* (Willd) is a perennial herb with a transversely quadrangular stem structure with silky hair covering it. It can grow up to 0.5 m to 2 m high. The Hausas of Northern Nigeria refer to it as "bambaro or maashayi," and it is common in North-East, East, and South-central Africa (Hamza *et al.,* 2020). *C. capitatum* is said to have been used traditionally in Nigerian medicine to treat a variety of conditions, including erectile dysfunction, fever, obesity, diabetes mellitus, diarrhoea, asthma, and hypertension (Habila *et al.,* 2018). Aqueous fresh leaf extract of *C. capitatum* had a hypoglycemic impact (Mostapha *et al.,* 2013), as well as immunomodulatory action, hypolipidemic activity, and antidiarrheal (Wang *et al.*, 2018). Many different pharmacological actions, including analgesic, hypothermic, diuretic, antioxidant, antibacterial, anticancer, and anti-inflammatory effects, are displayed by the chemicals and extracts that were extracted from *C. capitatum* (Yan *et al.*, 2018). The impact of *C. Capitatum* on albino rat renal function was examined by Adesina *et al*. in 2019, although the findings were unfavorable, having negative result (not active against the renal disease) (Adesina *et al.,* 2019). There is scarcity of report on the antibacterial activity of the pure isolate of *C. capitatum,* previous studies have focused on the crude extract. To the best of the authors' knowledge, there has never been a bioautography approach used to evaluate the antibacterial activity of *C. capitatum* plants. This would further highlight the plant's economic significance, given the public's apparent underutilization of these therapeutic plants. Hence, this study aims to determine the phytocompounds and antibacterial activity of the leaves of *C. capittatum*.

MATERIALS AND METHODS

Study Area

The study area, Rimi Local Government Area is situated three kilometres off the Katsina–Kano Federal Highway. Rimi is the Local Government Headquarters, located 22.2 kilometres from Katsina, the Capital of Katsina State. With a land area of 452 km^2, it is situated at latitudes 12° 51' 0'' to 13° 2'0'' North and longitudes 7° 49' 30'' to 8° 0' 30'' East (Suleiman *et al.,* 2017).

Sample Collection and Preparation

The roots of *C. capitatum* (Plate I) were collected from Rimi and Tudunkadir Community Area in the Rimi local government of Katsina State, Nigeria, during the Rainy Season in 2023.

Subsequently, the plant material was identified at the Department of Biological Sciences, Federal University Dutsin-Ma. The sample was washed, dried and grinded using mortar and a pestle. The sample was stored at room temperature in hygienic, airtight receptacles prior to other analyses.

Plate I: *Clerodendrum capitatum* leaves

Preparation of Extract

The pulverized powdered root sample (200 g) was extracted with n-hexane (400 ml), successively for seven days. Extract obtained were concentrated using the rotary evaporator and allowed to dry at room temperature.

Extract Yield (%)

A total weight of 200.00g powdered leaves of *C. capitatum* was used in the extraction of the plants material using n-hexane. The percentage yields of solvent extraction were obtained using the following formula:

%yields = Actual yield (weight of crude extract)/Theoretical yield (weight of plant material) × 100.

Phytochemical Screening

Preliminary Phytochemical Screening

A few milligrams of the n-hexane dried extract were dissolved and the solution obtained were subjected to phytochemical screening employing the standard screening test.

Test for Alkaloids

Few drops of Mayer's reagent were added to 1 mL of extract. A yellowish or white precipitate formed, indicate the present of alkaloids (Riji *et al.*, 2023).

Test for Tannins

To a portion of the extract, 3-5 drops of ferric chloride was added. A greenish-black precipitate indicates the presence of tannins (Riji *et al.*, 2023).

Test for Phenols

Two millilitres of 5% neutral ferric chloride solution were added to 1ml of extract, presence of phenolic compounds would be indicated by dark blue colour (Riji *et al.*, 2023).

Test for Flavonoids

A few drops of concentrated hydrochloric acid were added to a small amount of the extract of the plants material. Immediate development of a red colour indicates the presence of flavonoids (Riji *et al.*, 2023)

Test for Saponins

With a few ml of distilled water, 0.5 mg of extract was quickly shaken. For saponins, the production of foaming is a favourable sign (Riji *et al.*, 2023).

Test for Terpenoids

To 5 ml of the extract, 2 ml of chloroform and 3 ml of H_2SO_4 concentrated was added. Formation of a reddish brown ring confirms the presence of terpenoids.

Chromatography

Thin Layer Chromatography

To find the best solvent system that gives good separation between the compounds present and is suitable for column chromatography, the hexane extract was subjected to thin layer chromatography (TLC) using a variety of solvent systems (Hexane and ethyl acetate (4.5 ml:0.5 ml). Once the extract was detected on the TLC plate, it was left to dry. After drying, the plate was produced using the envisioned solvent system in an airtight chromatographic tank. Using ultraviolet light and ambient daylight, the developed chromatograms were air dried and viewed at (254nm and 366nm) wavelength.

Column Chromatography

Column chromatography was prepared silica gel (60H, Merck 7736) was mix with n-hexane, shaken well and poured in the column, and then sample (Hexane extract) was applied, silica gel was applied on the sample to avoid cracks. Solvent system containing 5%ethylacetate and 95% hexane was poured in to the column setup. 5ml fractions were collected up to about 30 fractions. The polarity of the solvent was increased to 10%EA and 90% hexane, fractions 30-100 were collected. TLC was run for all the fractions, and those having single spot, were merged together and put in a sample container for antimicrobial test, Bioautography and FT-IR analysis.

Antimicrobial Activity Test

The bacterial strains (*Salmonella typhi, Bacillus subtilis, Staphylococcus aureus, Bacillus megaterium, Klebseilla* spp*, Staphylococcus epidermidis, Escherichia coli, Pseudomonas aeruginosa, Proteus* spp*, Shigella* spp), from the culture plates were standardized by matching turbidity of insertion to 0.5M, McFarland standards which were then diluted in fresh broth (peptone water) and incubated at 37^{o}C for 24 hours. Thirty nine (39) gram of Mueller Hinton Agar (MHA) was prepared and 0.2 ml of each bacterial insertion were separated evenly. The dish was left on bench to set. Four wells, made by cutting a filter paper were put on the test plates. 0.2 g of the Isolate were measured using balance and dispensed into the clean and sterile test tube containing 2 ml of distilled water to obtained a concentration of 100 mg/ml, followed by transferring into another containing 1 ml to obtained 75 mg/ml, 50 mg/ml, 25mg/ml and 0 mg/ml, and stored for further analysis (Riji *et al.,* 2023). Ciprofloxacin (5 mg/ml) was set as control. The plates were then incubated at 37^{o}C for 24 hours and diameters of zone of inhibition were measured in mg/ml. Equal volume of extract and nutrient broth were mixed. 0.1ml of the standardized bacterial inoculum in the same tubes. The tubes were then incubated aerobically at 37^{o}C for 24 hours, positive control were equally set up. The tubes with the least concentrations and are able

to inhibit the bacterial growth were recorded as the MIC. The MIC were then sub-cultured on nutrient agar plates that contain no antibiotic, the lowest concentration that shows no growth of the bacteria was recorded as the MBC.

Bioautography

Bioautography was performed with culture of *salmonella typhi,* which show a good sensitivity to the isolated compound. Developed TLC were carefully dried for complete removal of the solvents and overlaid by agar seeded with an overnight culture of *Salmonella typhi*. The plates were incubated for 12 hours at 37^0C and then sprayed with an aqueous solution of 2 $mgml^{-1}$of p-nitrotetrazolium violet (Sigma). The areas of inhibition, white coloured were compared with retention factor (R_f) of the spot on the reference TLC plate (Nastro *et al.,* 2000).

Fourie Transform Infra-red (FT-IR)

The FTIR Spectrum of the active isolated compound was detected using shimadzu IR-470 plus, the spectra were also scanned in the 400 to 4000 cm^{-1} range and plotted as intensity versus wave number (Mohammad *et al.*, 2015).

RESULTS AND DISCUSSION

Extract Yield (%)

Total of 1.3 g was obtained as crude extract (0.63%), as shown in Table 1, representing the amounts of extract obtained after the extraction of the plant materials using n-hexane as solvents.

Thin Layer and Column Chromatography of the n-hexane Extracts

The TLC plate (6F 254, 60, Merck) 250 micrometre thick were developed, which separated components into a wide range of retention factor (R_f) values and the profile of crude extracts gives promising spots as shown in table 2, which show the presence of 8 spots on the TLC sheet (Plate II). The retention factor is the distance travelled by each spot divided by the distance travelled by the solvent system (mixture of hexane and ethyl acetate 4.5 ml: 0.5 ml respectively).

From the column chromatograph conducted about 100 fractions were collected out of which 5 fractions (number 23,24,25,26 and 27) after running TLC shows the same spots with the same R_f on their TLC sheets but which is not single. They were merged together and a small column was conducted, which resulted to the separation of the Isolate. Compound with R_f Value 0.35 was isolated (4.5 mg), it was viewed with UV lamp at (254nm and 366 nm) and we levelled it I1, as shown in the Plate III. This agrees with the result of Habila *et* al (2018),in which they isolate about 5 compounds from the same plant leave, thus this result differ from their findings due to the environmental and climatic differences.

Phytochemical screening result show the presence of alkaloids and terpenoids, while flavonoid, tannins, saponins, and phenolic compound are absent, this is in line with the result of Adeneye *et al.* (2008), were they found the presence of alkaloids in the leaves of *C. capitatum*. Also Ileke *et al*. (2018), found the presence of alkaloid in the hexane leaves extract of the same plant (*C. capitatum*). Triterpenoids was found in the phytochemical result of *C. capitatum* by Momoh *et al.* (2015) and Wang *et al.* (2018).

The result of the antimicrobial activity of the Isolated compound (I1), shown in table 4, exhibited a moderate activity against all the tested organisms, in all the concentrations (100-25 mg/L), except for *Bacillus magaterium* at 50mg/ and 25 mg/l, *Pseudomonas aeruginosa, Shigella spp,* and *Bacillus subtilis* all at 25 mg/l there is no activity. This indicates that, at higher concentrations there is higher activity than that in lower concentration, this is in line with the fact mention by Riji *et al*. (2023) that, antimicrobial activity is concentration dependant. Highest activity was recorded *against Staphylococcus epidermis* (zone of inhibition 17mm) in which the positive control (ciprofloxacin) has zone of inhibition of 24mm. the second highest activity was exhibited against *Salmonella thyphi* (zone of inhibition 16mm) compared with ciprofloxacin 27mm all the two higher activities were observed at 100mg/ml while the least/no activity was observed at 50mg/L against *Bacillus magaterium*, also at 25mg/mL against *Bacillus magaterium, Pseudomonas* spp*, Shigella* spp and *Bacillus subtilis*. This agrees with the result of antimicrobial activity of *C. capitatum* leaves isolated compounds, by Habila *et al.* (2018). The antimicrobial result of the isolated compound (I1) in this study indicates that the compound can serve as a lead for the development of drugs that can be effective against infectious diseases cause by the tested microorganisms.

Bioautography result also revealed clear zone of bacterial growth inhibition against *Salmonella thypi*.

The zone of inhibitions were less visible. From this result we can see that the isolated compound shows a well define inhibition band in correspondence with those of terpenes as mention in literature by Nastro *et al.* (2000).

Considering the FTIR spectroscopy co-ordinate pattern in Figure 1, the isolated compound (I1)with substantial antimicrobial activity, exhibited absorption at 2922cm-^{1} and 2847 cm-^{1} which is an indicator of hydrocarbon chassis, similar to the result obtained by Mohammad *et al.* (2015) and Riji *et al.* (2023). Another peak was observed at 1744 cm^{-1} which indicate presence of ketone functional group. Lack of peaks in the carbonyl (C=O) and hydroxyl (OH) stretching regions indicates the absence of polar compounds, as hexane(the extraction solvent) is generally effective in extracting non-polar substances (Riji *et al.,* 2023).

The presence of bioactive compounds with antimicrobial action, indicates the pharmacological activity of the *C. capitatum* leaves. Base on the medicinal uses of terpenoids and alkaloids presence in the phytochemical screening result (Table 3) and the observed zone of inhibitions from the antimicrobial activity test result (Table 4), indicates that the isolated compound from *C. capitatum* leaves has antimicrobial activity, which is the main aim of this study.

Table 1. Percentage Yield of Extracts

S/No	Extract	Yield (g)	% Yield (%)
1	n – hexane	1.3	0.65

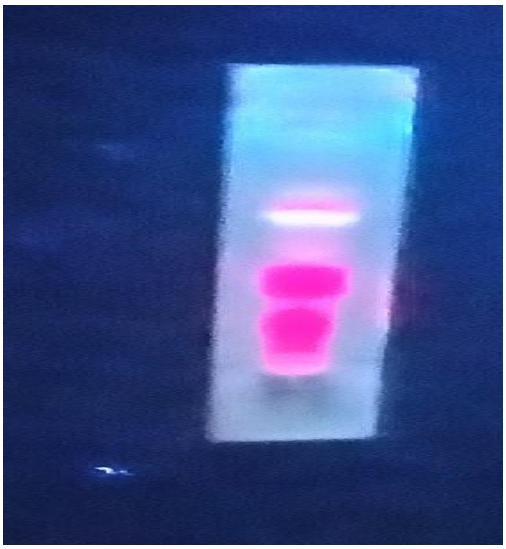

Plate II: Tlc of hexane extract of *C.capitatum*

Table 2. Retention Factors of crude n-hexane extracts

Component	Distance traveled by component (cm)	Distance traveled by solvent system (cm)	Retention Factor (Rf)
H_1	0.2	4	0.5
H_2	0.4	4	0.1
H_3	0.6	4	0.15
H_4	1.0	4	0.25
H_5	1.2	4	0.3
H_6	1.4	4	0.35
H_7	1.6	4	0.4
H_8	1.8	4	0.45

Table 3. Result of Phytochemical Screening of N-Hexane Extract

Secondary Metabolite	Hexane extract phytochemicals
Alkaloids	Present
Tannins	Absent
Phenols	Absent
Flavonoids	Absent
Saponins	Absent
Terpenoids	Present

Plate III. Isolate of Hexane extract from *C. capitatum*

Table 4. Results of Antimicrobial activity of various concentrations of *C. capitatum* isolate on test organisms

	Concentration (mg/mL)				Control
Organism	**100**	**75**	**50**	**25 L**	**Ciprofloxacin (5 mg/ml)**
Salmonellq typhi	16	11	9	7	27
Bacillus megaterium	10	8	ND	ND	21
Staphylococcus epidermidis	17	12	10	7	24
Klebseilla spp	14	12	9	8	27
Escherichia coli	13	10	8	8	31
Pseudomonas spp	11	8	8	ND	25
Proteus spp	14	10	8	8	27
Shigella spp	13	10	8	ND	25
Bacillus subtilis	11	8	8	ND	21
Staphylococcus aureus	15	11	8	7	27

KEY: ND= NOT DETECTED

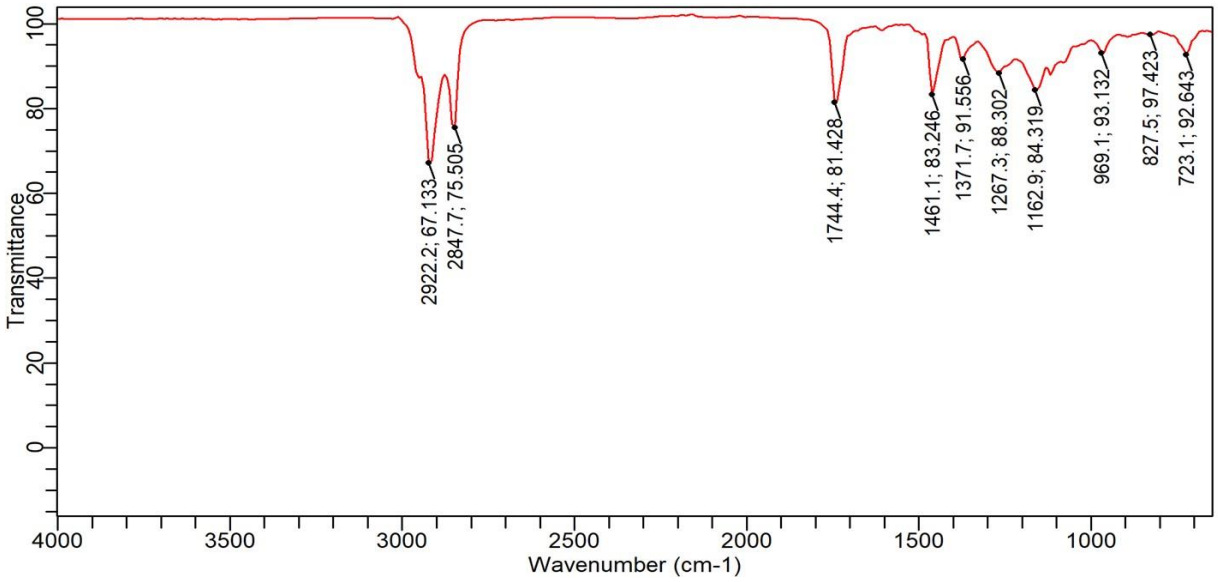

Figure 1. The FT-IR peaks

Table 5. FT-IR results

Peak number	Wavenumber (cm^{-1})	Intensity
1	723.10354	92.64345
2	827.46900	97.42318
3	969.10784	93.13245
4	1162.92941	84.31905
5	1267.29486	88.30152
6	1371.66032	91.55580
7	1461.11643	83.24629
8	1744.39411	81.42805
9	2847.68611	75.50528
10	2922.23286	67.13313

CONCLUSION

The isolated compound from *C. capitatum*, was studied and found to be active against some gram-positive and gram-negative bacteria. Bioautography analysis was also used to further evaluate this activity. Base on the medicinal uses of terpenoids and alkaloids presence in the phytochemical screening result, the observed zone of inhibitions from the antimicrobial activity test result, and the present of Ketone functional group from the result of FT-IR analysis of the isolated compound, indicates that the isolated compound from *C. capitatum* leaves has antimicrobial activity.

CONFLICT OF INTEREST

The authors declare no any conflict of interest.

FUNDING: This research work was funded by Tertiary Institution Trust Fund (TETFUND) Nigeria.

ACKNOWLEDGEMENT: We acknowledged the Almighty Allah, then the entire management of Federal University Dutsin-Ma, under the leadership of Professor Armaya'u Hamisu Bichi and all that supported us in conducting this research.

REFERENCES

: Riji, A. B., Rimi, A. D. and Sabo, A. S. (2023). "Isolation and Structural Elucidation of Antimicrobial Molecules from *Crinum ornatum (Aiton)* Rhizome of Dutsin-Ma". *Sahel Journal of Life Sciences FUDMA,* 1(1): 169-188.

Abubakar D. R. (2023). "Phytochemical Analysis of Components with Antimicrobial Activity from *Vernonia Kotschyana* (*Sch Bip.Ex Walp*) Rhizome

Extracts" A Masters Thesis Submitted to the Department of Chemistry Federal University Dutsin-Ma.

Adesina, J.M., Seyifunmi, O.E., Ofuya, T.I. and Adesina T.E. (2019). "Nephrotoxic and hephatotoxic effects of *Clerodendrum capitatum (wild) schumach thonn* and *phyllanthus fraternus schum* and *thonn (Euphorbiaceae)* extract used as food grains protectant on Albino rats". *Journal of biology and medicine.* 3(1): 008-013.

David, I.K.and Peter, A.F. (2018) "Bio efficacy of larvicidal and pupicidal properties of *Clerodendrum capitatum* and *Bridelia machrantha* leaves extracts against malaria vector *anopheleses*" *journal of biology and medicine.* 2(1): 007-011.

Epole, N. N., Eva, M., Domínguez, M., Amilcar, R., Joana, T., Vera, M. S., Paula, P., Maria, J. C. and Patrícia, R. (2020). "*Artemia species*: An Important Tool to Screen General Toxicity Samples". *Current Pharmaceutical Design. 26:* 2892-2908.

Habila, J. D., Isah, Y., Adeyemi, M. M., Ayo, R.G.,Ndukwe, G. I. and Momoh, H. (2018). "Antimicrobial and Antitubercular Activities of Bioactive Triterpenes from *Clerodendrum capitatum (Willd)* leaves". *J. Chem Soc. Nigeria*, 43, No. 2, pp 31 – 41.

Hamidu, U., Vivian, I., Helen, E.S. and Halima, A.U. (2017). "Comparative phytochemistry and invitro antimicrobial effectiveness of the leaf extracts of *Clerodendrum capitatum(verbenecea)* and *fiscus glumosa(moraceae)*". *Jornal of pharmacognosy and phytochemistry*. 6(6): 945-948.

Hamza M., Najma, I. and Mujtaba, S. A. (2020) "Anti-inflammatory and analgesic activities of *Clerodendrum capitatum* (Willd) Schum. & Thonn. (*Verbenaceae*) leaves" *journal of medicine and bioresources.* 17(1): 1-7.

Jin-Hui, W., Fei, L., Xiang-Dong, H., Yong, W. and Mao-Xing, L. (2018). "Traditionalusesandpharmacologicalproperties of *Clerodendrum* phytochemicals". *JournalofTraditionalandComplementaryMedicine.* 8: 24-38.

Mohammad R.P., Mohammed, M., Prashant, V.T. and Ashutosh, K. (2015). "Characterisation of bioactive compound isolated from Myrothecium spp, with UV, FTIR and HPLC analysis". *Indian j. pharm. Bio. Res,* 3(1): 1-5.

Mohammad, R.P., Mohammad, M., Prashant, V.T. and Ashutosh, K. (2015). "characterization of bioactive compound isolated from *Myrothecium spp*. With UV, FTIR, and HPLC analysis "*Indian journal of pharmaceutical and biological research.* 3(1): 1-5.

Momoh, H., Habila, J. D ., Ayo, R. G. and Ndukwe, G.I. (2015). "Clerodendrum capitatum(Willd) leave extracts inhibits bacteria, fungi and Mycobacterium bovis"Sch. Acad. J. Biosci. 3(4):386-391.

Mostafa A. F., Amira, S. and Khalil, E. (2013)." Phytochemical and Biological Studies of *Clerodendrum Glabraum* Leaves". *International Journal of Pharmacognosy and Phytochemistry.* 28(2): 2051-7858.

Nostro, A., Germano M.P., Angelo, V.D., Marino, A. and cannatelli M.A.(2000). "Extraction method and bioautography for evaluation of medicinal plant antimicrobial activity" *letters in applied microbiology.* 3(9): 384.

Nostro, A., Germano, V., D'angelo, V., Marino A. and Cannatelli, M.A. (2000). "Extraction method and bioautography for evaluation of medicinal plant antimicrobial activity".*Letters in applied microbiology.* 30: 379-384.

Pierre, M.(2023). "The genus *Clerodendrum* (*Lamiaceae*) in the flora of Central Africa (D.R. Congo, Rwanda, Burundi). *Phytotaxa.* 594(1): 001–042.

Suleiman, I. L. (2017). "Soil Fertility Management in the Rural Areas of Katsina State: A Case Study of Majen Gobir Village Rimi Local Government Area". *Ewemen Journal of Plant Genetics & Chemotaxonomy.* 2(1): 12-18.