**Journal of Biotechnology Research**
**ISSN(e): 2413-3256, ISSN(p): 2413-8878**
**Vol. 8, Issue. 1, pp: 1-6, 2022**
**URL:** https://arpgweb.com/journal/journal/16
**DOI:** https://doi.org/10.32861/jbr.81.1.6

**Academic Research Publishing Group**

**Original Research** **Open Access**

# Antibacterial Activities of Some Medicinal Plants on Common Multidrug-Resistant Bacterial Isolates from Some Patients Attending Federal Medical Center, Birnin Kebbi, Nigeria

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**Article History**
**Received:** 22 January, 2022
**Revised:** 23 February, 2022
**Accepted:** 1 March, 2022
**Published:** 4 March, 2022



## Abstract

Bacterial infections are among the major causes of morbidity and mortality worldwide. The development of drug resistance to most antibiotics by bacterial species are pandemic, these necessitate the urgent need to search for new antibacterial drugs from the natural sources. Hence this research was aimed at evaluating the antibacterial activity of some medicinal plants on the most commonly drug resistant bacterial isolates from some patients attending Federal Medical Center (FMC) Birnin Kebbi, Nigeria. An ethno-botanical survey was carried out within Zuru Emirate and three (3) plant samples (*Lonchocarpus laxiflorus* root, *Mitragyna inermis* root and *Lawsonia inermis* root) were selected based on their number of citations and limited number of reported researches on the antibacterial activities of their roots. The multidrug resistant bacterial strains (*Staplococcus aureus, Escherichia coli* and *Psuedomonas aeruginosa*) obtained from FMC, Birnin Kebbi, were taken from nutrient agar slant and sub-cultured in nutrient agar plates. The antibacterial activities of the root methanol extracts of the plants were evaluated using agar-well diffusion method. Tube dilution method was used to determine the minimum inhibitory concentration of each plant extract. The preliminary phytochemical screening of the extracts was conducted using standard methods. Alkaloids, Flavaniods, Steriods, Tannins, saponnins and Phenols, were detected in *L. laxiflorus* root methanol extract, Flavaniods were absent in *L. inermis* and Alkaloids in *M. inermis* root methanol extracts. The plant extracts and their combinations showed varying degrees of antibacterial activities on the test isolates. The root methanol extracts of *L. laxiflorus, L. inermis, L. laxiflorus/L. inermis, L. laxiflorus/M. inermis* and *L. inermis/M. inermis* roots presented significant increase ($p<0.05$) in the zone of inhibition against *S. aureus*. Extracts of *L. laxiflorus* root, *L. inermis* root and combination of *M. inermis* root were able to suppress the growth of *P. aeruginosa* at lower dose of 25mg/ml. The antibacterial activity revealed that, the extracts exhibited dose dependent effect. *L. inermis* root had the highest activity and least MIC value of 12.5mg/ml against all the tested isolates. Thus, this research justified the use of these plants in traditional medicine for the treatment of bacterial infections and can also be considered as potential sources for development of new antibacterial agents that may be more effective, safe and readily accessible than the current antibiotics.

**Keywords:** Bacteria; Antibacteria; Drug resistance; Medicinal plants; Phytochemicals.

## 1. Introduction

In spite of ever increasing efforts by researchers to discover medicinal potential of plants [1], the potentials of many higher plant still remain unexplored [1]. Likewise efforts are mostly channeled on the leafy or bark of the

plants in discovering new drugs and the root parts are a times neglected. Bacterial infections are treated with wide range of antibiotics, most of which are either too expensive or not readily accessible by many people, particularly those living in rural area [2]. That is why people living in rural communities still rely more on traditional medicines for treating infections. The development of resistance to many antibiotics by bacteria is an issue of major concern [3]. The speed at which the problem is growing makes the outlook for the use of antimicrobial drugs in the future very uncertain [4]. This necessitate the need to search for antimicrobial drugs from natural sources [5]. Plants are used medicinally in different countries as a source of many potent and powerful drugs. Most of developing countries have adopted traditional medical practice as an integral part of their culture [6]. Plant materials are present and have provided models for 50% of western drugs [7]. Medicinal plants generally contain a number of compounds which may potential natural product for the treatment of common bacterial infections [8]. These combination of complex phytochemicals acting by different mechanisms, makes it difficult for pathogens to develop resistance [9]. The primary benefit of using plant medicine is that there are relatively safer and cheaper than their synthetic counterpart [1]. Researchers are increasingly turning their attention to herbal products in discovering new leads to develop better drugs against MDR strains [10]. The present study was designed to investigate the antibacterial potentials of the methanol root extracts of the widely acclaimed medicinal values of *L. laxiflorus* and *M. inermis* against some common MDR strains isolated in Birnin Kebbi.

# 2. Materials and Methods

## 2.1. Plant Samples Collection and Authentication

The *Lonchocrpus laxiflorus* and *Mitragyna inermis* roots were collected within Bena town, Nigeria. The samples were identified and authenticated in the Department of Plant Science and Biotechnology, Kebbi State University of Science and Technology, Aliero, Nigeria.

## 2.2. Multidrug Resistant Bacteria

The clinically isolated multidrug resistant bacteria (*Staplococcus aureus, Psuedomonas aeruginosa* and *Escherichia coli*) were obtained from Federal Medical Center Birnin Kebbi, Nigeria. Sensitivity test was conducted to confirm their multidrug resistance.

## 2.3. Preparation of Plant Samples

The plant root samples were cleaned and air dried at ambient temperature. The completely dried samples were grinded with mortar and pestle to coarse powder.

## 2.4. Plant Samples Extraction

A 150g of the dried powder of each plant sample was dissolved in 500ml 0f 85% methanol. The mixture was gently stirred, it was then tightly covered with cotton wool and aluminum foil and was allowed to stand for 48 hours at ambient temperature. The sample was decanted and filtered through muslin cloth and Whatman filter paper No.1. The filtrate obtained was allowed to evaporate at ambient temperature with continuous weighing until a constant weight was obtained. The plant extracts were used as dose mg/ml concentration.

## 2.5. Media Preparation

A 28g of nutrient agar was dissolved in 1L of distilled water in a conical flask. The mixture was heated using a hot plate until all the content were completely dissolved. It was then sterilized using autoclave at 121$^{o}$C for 15 minutes and allowed to cool at ambient temperature, it was then poured in petri dishes and allowed to solidify [10].

## 2.6. Culture and Maintenance of Test Organisms

The isolates (*Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli*) were taken from nutrient agar slants and sub-cultured on nutrient agar plates. The plates were incubated at 37 $^{o}$C for 24 hours to get a sub-culture of the isolates.

## 2.7. Preparation of Inoculums

After the sub-culturing, to prepare the bacterial inoculums, the sub-cultures were inoculated on fresh nutrient agar plates using sterile cotton swabs and incubated at 37 $^{o}$C for 24 hours**.** The pure cultures on the nutrient agar plates were used as the inoculums [11].

## 2.8. Antibiotic Sensitivity Test

Nutrient agar media was poured into 100mm petri-dishes and allowed to solidify. Bacterial inoculum was prepared by diluting the agar culture to match the 0.5 Mcfarland turbidity standard. A sterilized swap was used to collect the culture, excess culture was removed by gently pressing the swap against the surface of the tube. The swap was then streaked across the nutrient agar plates to form a bacterial lawn, in order to achieve a uniform growth the swap was streaked in the agar plate in one direction, rotated at 120$^{o}$ it was streaked again rotated at another 120$^{o}$. Flame sterilized forceps were used to pick and gently pressed antibiotic disc into the plates. The plates were then incubated at 35 $^{o}$C overnight. The antibacterial activity was interpreted by a clear zone around a disc which was measured in mm with a ruler [12].

## 2.9. Anti-bacterial Activity Screening of the Selected Plant Extracts

Agar well diffusion method according to Ericsson [12] was used to evaluate antibacterial activity of the plant extracts. The agar plates were inoculated with a volume (1ml) of the bacterial inoculum, which was spread over the entire surface. Holes of 6mm in diameter were aseptically dug using a sterilized cork borer and a volume of 100µl of 100, 75, 50 and 25mg/ml extract were added to the respective wells. The plates were then incubated at 35°C overnight. The clear portion around the wells were measured as zones of inhibition [13].

## 2.10. Determination of Combined Effect

A 40g of each of the individual powered plant was mixed together making 120g, and was soaked in 85% methanol for 48hours. The mixtures were filtered using muslin cloth, and then re-filtered using Whatman filter paper. The solvent was then allowed to evaporate in a water bath [14].

## 2.11. Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of each plant extract was determined according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.

Tubes dilution method was used. Serial dilutions of the extracts were made in a liquid medium which is not inoculated with standardized number of organisms and incubated for prescribed time. The lowest concentration (highest dilution) of extract preventing appearance of turbidity is considered to the minimal inhibitory concentration (MIC) [15]. At that dilution the extract is bacteriostatic. For each extract, 9 test-tubes were numbered and sterilized in an autoclave for 15 minutes. A 2.0 ml of the extract solutions were added to the first test-tubes. 1.0 ml from the first test-tubes was transfer to the second tubes. Using a separate pipette the content of the second tubes was mixed and 1.0 ml was transfer to the third tubes. Dilution continues up to tube number eight, certainly changing pipettes between tubes to prevent carryover of the extract on the external surface of the pipette. A 0.1 ml was then removed from tube eight and discarded. No extract was added in tube 9, it served as the control. Several colony of the test culture were suspended into 5.0 ml of nutrient agar broth to give a slightly turbid suspension. The suspension was aseptically diluted by pipetting 0.2 ml of the suspension into 40ml of nutrient agar broth. 1.0 ml of the suspension was added to each tube. The inoculated tubes were incubated in an ambient air incubator overnight. After incubation, the amount of growth in the tubes containing extracts was compared with the amount of growth in control tubes (tubes with no extracts) to determine the growth end point. Comparison was based on the turbidity, the higher the turbidity the lesser the antimicrobial activity.

## 2.12. Preliminary Phytochemical Screening of the Plant Extracts

Five grams (5g) from the extracts were dissolved in 40 ml of distilled water and then subjected to the phytochemical screening to test for the presence of flavonoids, phenols, tannins, saponins, alkaloids and steriods. The phytochemicals were investigated according to common described phytochemical methods.

# 3. Results

**Table-1.** Antibiotic Sensitivity Profile of the Test Bacteria

| Bacterium | Antibiotic | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | PG | AM | CPX | E | APX | AMP | CH | AZM | AT | SXT |
| *S. aureus* | - | - | + | + | - | - | - | - | - | + |
| *E. coli* | - | + | + | - | - | - | - | - | + | - |
| *P. aeruginosa* | - | - | + | + | - | - | - | + | - | - |

PG=Defloxacine, AM= Amoxicillin, CPX=Ciprofloxacin, E=Erythromycin, APX=Ampiclox AMP= Ampicillin, CH Chloramphenicol, AZM=Azithromycin, AT=Augmentin SXT= Septrin. +=Susceptible, -=resistance. All the test isolates were found to be susceptible to ciprofloxacin

**Table-2.** Antibacterial Activity of *L. laxiflorus* root methanol extract on the test isolates

| Extracts (mg/ml) | *S. aureus* | *P. aeruginosa* | *E. coli* |
|---|---|---|---|
| 25 | 9.67±0.33 | 9.33±0.67 | 0.00±0.00 |
| 50 | 13.67±0.33 | 11.33±0.88 | 0.00±0.00 |
| 75 | 17.33±0.33 | 14.67±0.67 | 0.67±0.67 |
| 100 | 20.00±.0.58 | 18.33±0.88 | 13.67±2.73 |

Values are presented as Mean ± SEM of triplicates.

**Table-3.** Antibacterial activity of root methanol extract of *M. inermis* on the test isolates

| Extracts (mg/ml) | Zone of Inhibition (mm) | | |
|---|---|---|---|
| | *S. aureus* | *P. aeruginosa* | *E. coli* |
| 25 | 0.00±0.00 | 10.33±0.33 | 0.00±0.00 |
| 50 | 0.00±0.00 | 12.33±0.88 | 0.00±0.00 |
| 75 | 0.00±0.00 | 16.33±0.33 | 8.33±0.88 |
| 100 | 0.83±0.83 | 21.67±0.88 | 10.67±0.33 |

Values are presented as Mean ± SEM of triplicates.

**Table-4.** Combined effect of the mixed methanol extracts on the test isolates

| Extracts (mg/ml) | Zone of Inhibition (mm) | | |
|---|---|---|---|
| | ***S. aureus*** | ***P. aeruginosa*** | ***E. coli*** |
| 25 | 0.00±0.00 | 2.67±1.45 | 1.67±1.67 |
| 50 | 0.00±0.00 | 11.00±0.58 | 5.67±2.85 |
| 75 | 12.67±0.67 | 12.73±0.37 | 12.67±0.33 |
| 100 | 16.00±1.00 | 21.00±5.77 | 18.00±0.58 |

Values are presented as Mean ± SEM of triplicates.

**Table-5.** Minimum inhibitory concentration of the plants root methanol extracts on *Staphylococcus aureus*

| Extracts | MIC (mg/ml) |
|---|---|
| *L. laxiflorus* | 25 |
| *M. inermis* | 100 |
| *L. laxiflorus/ M. inermis* | 100 |

**Table-6.** Minimum inhibitory concentration of the plants root methanol extracts on *Pseudomonas aeruginosa*

| Extracts | MIC (mg/ml) |
|---|---|
| *L. laxiflorus* | 25 |
| *M. inermis* | 25 |
| *L. laxiflorus/ M. inermis* | 25 |

**Table-7.** Minimum inhibitory concentration of the plants root methanol extracts on *Escherichia coli*

| Extracts | MIC (mg/ml) |
|---|---|
| *L. laxiflorus* | 100 |
| *M. inermis* | 100 |
| *L. laxiflorus/ M. inermis* | 25 |

**Table-8.** Qualitative Phytochemical Composition of the Extracts

| Phytochemicals | *L. laxiflorus* | *M. inermis* |
|---|---|---|
| Saponins | + | + |
| Tannins | + | + |
| Flavonoids | + | + |
| Alkaloids | + | - |
| Phenols | + | + |
| Steroids | + | + |

+ = Present, - = Not detected

# 4. Discussion

In the search to counter resistance to antibiotics among bacterial strains that has become a global threat, an ethno botanical survey was conducted in Zuru Emirate of Kebbi State. Being naturally blessed with suitable climate and fertile soil, Zuru is a rich flora of indigenous plants. It is well known throughout the state for expertise in herbal medicine preparation for treatment of diseases and healing injuries. The antibacterial activities of the selected plants were investigated on three clinically isolated resistant bacterial strains commonly found in patients attending Federal Medical Center (F.M.C) Birnin Kebbi. The strains involves one gram positive bacterium (*Staphlococcus aureus*) and two gram negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*). The ethno medicinal importance of the selected plants has been reported in various indigenous system of fork medicine and scientific documents [5, 16]. The plants have been widely used in the treatments of diseases such as syphilis, typhoid fever, jaundice, Acquired Immune Deficiency Syndrome (AIDS), skin infections [17-20]. The powder /paste forms of the root, stem and leaf extracts of the plants have also been reported to be used in treatment of asthma, pains, blood, skin and lung diseases, cataract, malaria and epilepsy [21-23]. The following phytochemicals have been associated with the plants: alkaloids, flavonoids, saponins, tanins, phenols, terpenoids, carbohydrates, glycosides, sterols [24-26]. In order to provide more scientific justification for the utilization of these plants this study was carried out to further prove some of these ethno medicinal claims.

The antibacterial activity of the methanol root extracts of *Lonchorcapus laxiflorus* and *Mitragyna inermis* presented in tables 2-4 revealed *Lonchocarpus laxiflorus* to have (9.67 at 25mg/ml, 13.67 at 50mg/ml, 17.33 at 75mg/ml and 20.00 at 100mg/ml. *Mitragyna inermis* shows activity only at 100mg/ml which is 0.83. *Lonchocarpus laxiflorus* is effective against *Staphlococcus aureus*. This result support previous findings by Mubiu [27] showing that, the root bark extract of *Lonchocarpus specie* tested against *Staphlococcus a*ureus to be an effective antimicrobial agent [27]. Likewise, Fiot, *et al.* [28] showed in their studies that the ethanol leaf extract of *Mitragyna inermis* have a wider range of zone of inhibitions when tested against *Staphlococcus aureus*, [28] however this may not support the present findings, in this research the methanol root extract of *Mitragyna inermis* is less effective against *Staphlococcus aureus*, although this could change if the extract concentration is increased. A report published by Wakirawa, *et al.* [29] may likely support the present findings, it reveals the methanol leaf extract of

*Mitragyna inermis* root to be less effective against *Staphlococcus aureus* compared to the other tested organisms [29]. The variations between the previous and present result may be likely due to the method and/or solvent used in extraction as well as the part of the plant used. The combination effect of the extracts (*Lonchocarpus laxiflorus/Mitragyna inermis* root) against *Staphlococcus aureus* shows activity of 1.67 at 25mg/ml, 5.73 at 50 mg/ml, 12.67 at 75mg/ml and 18.00 at 100mg/ml. *Lonchocarpus laxiflorus* gives (9.67 (25mg/ml), 13.67 (50mg/ml), 17.33 (75mg/ml) and 20.00 (100mg/ml) *Mitragyna inermis* shows activity only at 100mg/ml which 0.83. at 75mg/ml and 15.33 at 100mg/ml concentrations against *E.coli*. The combined extracts (*Lonchocarpus laxiflorus/Mitragyna inermis* root) yielded 1.67, 2.33, 9.66 and 11.97 at 25, 50, 75 and 100mg/ml.50, 75 and 100mg/mg respectively. The antibacterial activities of *Mitragyna inermis* against *Pseudomonas aeruginosa* gives (10.33, 12.33, 16.33 and 21.67), *Lonchocarpus laxiflorus* have the least activity with (9.33, 11.33, 14.67 and 18.33), at 25, 50, 75 and 100mg/ml respectivily. The combination effect of the extracts against *Pseudomonas aeruginosa*, yielded an activity of 2.67, 11.00, 12.73 and 21.00 at 25, 50, 75 and 100mg/ml respectively. The highest activity of each extract could be seen in 100mg/ml concentrations this support previous findings by Aremu and Adekoya that the antimicrobial activity have a direct relationship with increasing the extract concentration [30]. From the result obtained, the antibacterial activities of each extract varies with regards to the isolate. (*Lonchocarpus laxiflorus* root) is more effective against gram positive *Staphlococcus aureus* followed by *Psuedomonas aeruginosa* and then *Escherichia coli*. While (*Mitragyna inermis* against gram negative *Psuedomonas aeruginosa* and *Escherichia coli* than gram positive *Staphlococcus arueus.* These present findings may not support Masoodi, *et al.*, and other reports that viewed gram positive bacteria as more sensitive bacteria towards plant extracts than gram negative bacteria [31]. In line with previous findings that viewed synergisms to be more effective than individual extract, results from this research also shows increase in activity when the extracts are combined.

The MIC of the root extract of *L. laxiflorus* on *S. aureus, P. aeruginosa* and *E. coli* is 25, 25 and 100, that of *M.inermis* is 100, 25 and 100 respectivily. The combined extracts (*L. laxiflorus/ M.inermis*) gives 100, 25 and 25 against *S. aureus, P. aeruginosa* and *E. coli*.

The six phytochemicals investigated which are in table 8 were all found to be present in *L. laxiflorus*, but alkaloids was not detected in *M.inermis*. From the results it is evident that the antibacterial activity of the methanol root extracts may be due to the presence of these phytochemicals, likewise the variation of activities exhibited by the root extracts may be due to concentration of the constituents among the and difference in mode of action against tested isolates.

## 5. Conclution

The studied plants presented promising antibacterial activities as there is significantly increased efficacy between the test plants and the control. This research justified the use of these plants by traditional healers for the treatment of bacterial infections. It has also provided useful information for discovering new compounds with better activity and more effective against resistant bacteria than the current available antibiotic drugs.

## References


[1] Sani, I., 2014. "Application of medicinal plants to overcome antibiotic resistance in some selected multi-drug resistant clinical isolates. ." *Research and reviews J. of Pharmaco and Phytochem*, vol. 2, pp. 48-52.

[2] Sengupta, S., Chattopadhyay, M. K., and Grossart, H. P., 2013. "The multifaceted roles of antibiotics and antibiotic resistance in nature." *Front Microbiol*, vol. 12, p. 47.

[3] Odugbemi, T., 1981. "The use and abuse of antibiotics." *Nig. Med. Pract.*, vol. 1, pp. 4–8.

[4] Sani, I., Umar, R. A., and Hassan, S. W., 2018. "Potential alternatives for resolving bacterial antibiotic resistance." *Eur. J. Pharm. Med. Res.*, vol. 5, pp. 25-34.

[5] Sani, I., Bello, F., and Abdul-Kadir, D., 2014. "Phytochemical screening and antibacterial activity of Allium sativum, Calotropis procera, Acacia nilotica and Mitracarpus scaber mixed hexane extracts." *World J. Pharmaceut. Res.*, vol. 3, pp. 142-149.

[6] Sofowora, A., 2008. *Medicinal plants and traditional medicine in Africa*. 3rd Edition ed. Ibadan Nigeria. (Google Schoolar): Spectrum Books Limited.

[7] Ahmed, N., 2012. "Medicinal plants and their importance as an alternative medicine." Available: www.Researchgate.net/profile/mdNasirAhmed

[8] Frantisek, S. and Vaclav, J., 1976. *Medicinal Plants*. Artia ed. Prague: Illustrated Atlas. p. 247.

[9] Aiyegoro, A. O. and Okoh, A. I., 2009. "Use of bioactive plant products in combination with standard antibiotics: Implications in antimicrobial chemotherapy." *Journ. of Med. Plants Res.*, vol. 3, pp. 1147-1152.

[10] Khan, R., Islam, B., Akram, M., Shakil, S., Ahmad, A., Anazir, A. S., Saddiqui, M., and Khan, A. U., 2009. "Antimicrobial activity of five herbal extracts against multi-drug resistant (mdr) strain of bacteria and fungus of clinical origin." *Mol.*, vol. 14, pp. 586-56.

[11] Sani, I., Bello, F., and Abdul-Kadir, D., 2016. "Phytochemical screening and antibacterial activity of Allium sativum, Calotropis procera, Acacia nilotica and Mitracarpus scaber mixed hexane extracts." *World J. Pharmaceut. Res.*, vol. 3, pp. 142-149.

[12] Ericsson, B. H., 1960. "The paper disc method for determination of bacterial sensitivity to antibiotics studies on the accuracy of the technique." *Scandinavian Journal of Clinical and Laboratory Investigation*, vol. 12, pp. 408-413.

[13] Pelczar, M. J., Chan, E. C. S., and Krieg, N. R., 1993. *Microbiology: Concepts and applications* vol. 5. New York, USA: McGraw Publication. pp. 25-34.

[14] Habbal, O. A., Al-Jabri, A. A., El-Hag, A. H., Al-Mahrooqi, Z. H., and Al-Hashmi, N. A., 2005. "In-vitro antimicrobial activity of Lawsonia inermis Linn (henna). A pilot study on the Omani henna." *Saudi Med. J.*, vol. 26, pp. 69-72.

[15] Brock, A., Herzfeld, T., Paschke, R., Koch, M., and Drager, B., 2006. "Pharmacognosie, phytochimie, plantes médicinales." *Phytochemistry,* vol. 67, pp. 2050-2057.

[16] Ali, M., 2008. *Isolation of natural products. Pharmacognosy (pharmacognosy and plant cultivation)*. Eds. Satish Kumar Jain for CBS Publishers and Distributors.

[17] Chaudhary, G., Goyal, S., and Poonam, P., 2010. "Lawsonia inermis linneaeus. Phytopharmacological review." *International Journal of Phamaceutical Sciences and Drug Reseach,* vol. 2, pp. 91-98.

[18] Chukwu, O. O. C., Odu, C. E., Chukwu, D. I., Hafiz, N., Chidozie, V. N., and Onyimba, I. A., 2011. "Application of extracts of Henna (Lawsonia inamis) leaves as a counter stain." *Afr. J. Microbiol. Res.,* vol. 5, pp. 3351-3356.

[19] Pelter, A. and Amenechi, P. I., 1969. "Isoflovonoid and pterocarpinoid extractives of Lonchocarpus laxiflorus." *Journal of the Chemical Society C: Organic,* pp. 887-896. Available: doi: 10.1039/J39690000887

[20] Placide, M. T., 2020. "Phytochemistry and pharmacological review of mitragyna inermis (willd) kuatze (rubiancece)." *Jounl. of Phamacol.and Phytochem. JPP,* vol. 9, pp. 22-30.

[21] Azas, N., Laurencin, N., Delmas, F., Di, G. C., Laget, M., and Timon-David, P., 2002. "Synergistic in vitro antimalarial of plant extracts used as traditional herbal remedies in Mali." *Parasitol. Res.,* vol. 88, pp. 165-171.

[22] Barges, A. R., Rodriguez, L. M., and Waterman, P. G., 2000. "Favonoids from the stem bark of Lonchocapus xuul." *Phytochemisry,* vol. 54, pp. 611-614.

[23] Chevalier, J., Pages, J. M., Eyrand, A., and Mallea, M., 2000. "Membrane permeability and modification are involved in antibiotic resistance." *Biochem. Biophys. Commun,* vol. 274, pp. 496-499.

[24] Amegnona, A. and Messanvi, G., 2009. "hepatoprotective effect of lonchocarpus sericeus leaves in CCl4 - induced liver damage." *Journal of Herbs, Spices and Medicinal Plants,* vol. 15, pp. 216-226.

[25] Asase, A. T., Kokubun, R. J., Simmonds, A., Yeboah, A. O., and Odamtten, G. T., 2008. "Chemical constituent and antimicrobial activity of medicinal plants from Ghana: Cassia sieberiana, Haematostaphis barteri, Mitragyna inermis and pseudomonas kotschyi." *Phytother. Res.,* vol. 22, pp. 1013- 1016.

[26] Muktar, M., Adamu, H. M., and Falalu, M. Y., 2006. "GC-MS analysis and identification of constituents present in the root extract of mitragyna inermis." *Journal of Pharmacognosy and Phytochemistry,* vol. 5, pp. 17-20.

[27] Mubiu, J. K., 2018. *Antimicrobial activity and constituent of the root, bark of lonchocarpus eriocalys*. University of Nairobi: M.sc Thesis Submitted to the Department of Pharmacology, School of Pharmacy.

[28] Fiot, J., B., B., and Boyer, L., 2005. "HPLC quantitation of uncarine d and the antiplasmodial activity of alkaliods from leaves of mitragyna inermis (wild) o. Kuntze." *Phytochem. Anal.,* vol. 16, pp. 30-33.

[29] Wakirawa, J. H., Yawate, Zakama, U. E., SMuaz, G., and Madu, S. J., 2013. "Phytochemical and antibacterial screening of the methanol leaf extract of mitragyana inermis (Wild O. Ktze Rubiaceace)." *International Journal of Pharmaceutical Research and Innovation,* vol. 6, pp. 1-6.

[30] Aremu, O. I. and Adekoye, A. G., 2009. "Phytochemical and antibacterial screening of the root extract of Carica papaya." *Int. J. Pharm. Sci.,* vol. 1, pp. 13-17.

[31] Mosoodi, M. H., Ahmad, B., Zargar, I. M., Khan, S. A., and Singh, P., 2008. "Antibaterial Activity of Whole Plant Extract of Marribium vulgare." *Afri. J. Biotechnol.,* vol. 7, pp. 086-087.