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# Bioactive Constituents and Antibacterial Screening of Two Nigerian Plant Extracts Against Selected Clinical Bacteria

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

**Abstract**

**Background:** The growing desires to combat antibiotic resistance among pathogenic bacteria necessitate the need to search for new antimicrobials agents from other sources such as plants.

**Objectives:** The present study investigated the antibacterial activities and bioactive components of *Nymphaea lotus* and *Spondias mombin* against selected clinical bacteria

**Materia**l **and Method**s: Extracts of *N. lotus* and *S. mombin* were prepared by 72 hours maceration in 70% methanol. The antimicrobial susceptibility testing (AST) of *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Salmonella typhi*, *Staphylococcus aureus*, *Citrobacter freundi* and, *Klebsiella oxytoca* against the two extracts was carried out by disk diffusion method while minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) was by agar-well dilution and broth dilution method, respectively. The bioactive compounds of the plants were identified by Gas Chromatography-Mass Spectrometry (GC-MS) analysis.

**Result**s: Extracts of *N. lotus* showed better antimicrobial activities than *S. mombin* against all the clinical bacterial isolates with an MIC range of 3.13 – >12.5mg/mL compared to *S. mombin* with MIC range of 6.25 – >12.5mg/mL. The GC-MS results revealed the presence of 21 and 25 compounds for *N. lotus* and *S. mombin* respectively. Benzoic acid derivatives were in abundance in both plants with approximately 71.5% and 82.1% in *N. lotus* and *S. mombin* respectively.

**Conclusion**s: The findings from this study provided further evidence on their ethno-botanical claims and additional information on the potentials of the studied plants as effective medicinal plants with antimicrobial activity against clinical bacteria. This highlights the need for continuous exploration of medicinal plants for novel compounds with better antimicrobial property as option for the treatment of resistant bacterial infection

**Keywords**: *Nymphaea lotus*, *Spondias mombin*, Bioactive components, Antimicrobials

## INTRODUCTION

Emergence of multidrug resistant organisms (MDRO) is an increasingly global pressing concern. The enthusiasm of antibiotics in the treatment of infectious diseases is gradually losing its euphoria due to the threat of MDROs which are signs of approaching post-antibiotic era (Fowler *et al*., 2014).Resistant bacteria can significantly increase hospital stay and increase the cost of medication due to complications in their treatments in critically ill patients, especially in surgery and intensive care unit (Livermore, 2012; Nordmann*et al*., 2012). Bacterial isolates capable of hydrolyzing all available antibiotics have also been described (Hrabák *et al*., 2011;Tzouvelekis*et al*., 2012). MDROs are responsible for increasing morbidity and mortality rate. However, scientific quest to arrest the situation with alternative means such as production of newer antibiotics (Devasahayam *et al*., 2010), the use of bacteriophage (Matsuzaki *et*

*al*., 2005) and immune-enhancing strategies (Spellberg *et al*., 2013) are either slow or yet to be accepted as a practical adjunct to the current antibiotics.

The use of plant as a remedy for treatment of various ailments including infectious diseases has been on for centuries (Azaizeh *et al*., 2003; Saadabi, 2007). These natural products serve as prototypes for the development of more active antimicrobial compounds with less toxicity and with prospects of a large variety of drugs for human consumption (Lewis and Ausubel, 2006). Plant derived medicines are widely used because they are relatively safer, readily available and cheaper than the synthetic alternatives (Iwu *et al*., 1999). In Nigeria and other countries, various plants have been reported to be effective against several pathogenic microorganisms (Adeniyi *et al*., 2009; Djeussi *et al*., 2013). For instance, the edible flower of *Sesbania grandiflora* was shown to have inhibitory effects against *Staphylococcus aureus, Shigella flexneri, Salmonella typhi, Esherichia coli and Vibrio cholerae* at lower concentrations (Minimum Inhibitory Concentration (MIC) range 0.013 – 0.25mg/mL) (China *et al*., 2012). Djeussi *et al*. (2013) reported that five out of seven Cameroonian plants used in their study exhibited varying degrees of antibacterial activities against twenty-seven bacteria tested. Two species of *Eucalyptus* plant (*Eucalyptus camaldulensis* and *Eucalyptus torelliana*) from Nigeria were shown to exhibit a potent activity against *Helicobacter pylori* as reported by Adeniyi *et al*. (2009). The leaves and roots of *Plectranthus barbatus* were reported to be traditional remedies in India for various medical conditions such as heart and lung diseases, asthma, insomnia, muscle spasm, convulsions, digestive complaints and skin disease (Foster and Johnson, 2006).

*Nymphaea lotus* and *Spondias mombin* are commonly used plants in Nigerian traditional medicine and it has been used in the treatment of fever, skin diseases, cancer, gonorrhea and bronchitis (Elegami *et al*., 2003). *S. mombin* is a fructiferous tree native to Nigeria, Brazil and other tropical forest of the world. The plant is known for its folkloric remedy for diarrhea, dysentery, relief from stomach ache, eye and throat inflammations. Its non-medical uses include production of ice creams and other variety of food consumption. Since there has been a growing interest in the investigation and discovery of new antimicrobial agents with promising activities against the imminent threat of multidrug resistant bacteria, there is an urgent need for constant identification of plants with novel bioactive phytochemicals that are active against MDROs. This study evaluated the bioactive components of *N. lotus* and *S. mombin* and determined the antimicrobial activities of the extracts against clinical bacteria.

## MATERIALS AND METHODS

### Collection and extraction of plant materials

Fresh leaves of *N. lotus* were collected at Eleyele dam, Ibadan in Oyo State while *S. mombin* leaves were collected within the premises of University of Ibadan, Ibadan, Nigeria. Both plants were authenticated at the herbarium of the Department of Botany, University of Ibadan with voucher specimen numbers (UIH-22349) and (UIH 22350) respectively. The plants were dried in a well aerated environment at room temperature and then pulverized into coarse powder using domestic electric blender. Cold extraction was performed on the two plant materials by weighing 500g each of the pulverized coarse powder then soaked in 5 L of 70% v/v methanol for 72 hours with constant shaking at room temperature and filtered using Whattman's No. 1 filter paper (Yahaya *et al*., 2012). The filtrates obtained were concentrated by complete evaporation of the solvent under reduced pressure in a vacuum using a rotary evaporator at 55$^{\circ}$C to obtain dried powdered crude extracts (Yahaya *et al*., 2012).

### Collection and authentication of bacterial isolates

The seven clinical bacterial isolates collected from the Laboratory benches of Department of Microbiology and Parasitology, University College Hospital (UCH), Ibadan, Nigeria were used in this study. The identities of the isolates were confirmed using conventional biochemical and sugar fermentation test. The bacteria included: *Escherichia coli, Klebsiella pneumoniae, Enterobacteraerogenes, Salmonella typhi, Staphylococcus aureus, Citrobacter freundii* and *Klebsiella oxytoca.* The laboratory record showed that the *E. coli* and *K. pneumoniae* were isolated from patients with urinary tract infection (UTI). *S. aureus* was isolated from furuncle, *S. typhi* from blood sample of a bacteremia patient while *E. aerogenes*, *K. oxytoca* and *C. freundii* were nosocomial pathogens. The bacterial isolates were maintained at 4$^{o}$C on Nutrient agar slants.

### Antibiotic susceptibility test of the bacterial isolates

All the bacterial isolates used in this study were tested against standard antibiotics using the disc diffusion technique (Kirby-Bauer, 1996) on Mueller Hinton agar. The antibiotics tested included ceftazidime (30 µg), cefuroxime (30 µg), gentamicin (10 µg), ofloxacin (5 µg) and amoxicillin/clavulanate (20/10 µg). The zones of inhibition were measured and compared with the breakpoints specified by the Clinical and Laboratory Standards Institute guidelines (CLSI, 2014). *Pseudomonas aeruginosa* ATCC 27853 was used as the control strain.

**Antibacterial assessment of the plants' extracts against selected isolates by disk-diffusion method**

**Preparation of disks impregnated with extract concentrates**

Paper disk used as vehicle for the application of the plant extract against the bacterial isolates was prepared by the method described by Velmonte *et al.* (1988) with slight modifications. Whattman's filter paper No. 3 were cut into several 7mm diameter disk using a paper punch then sterilized and further impregnated with 20µL of different concentrations of the methanolic extracts and allowed to dry for 2 hours before use.

**Antibacterial susceptibility test of the extracts**

A colony of an18-24 hours culture of each bacterium adjusted to 0.5 McFarland standard which corresponds to 1.5 x $10^8$CFU/mL was spread evenly on Mueller Hinton agar surface (Difco, USA) using sterile swab sticks dipped into the bacteria suspension. With the aid of a sterile forcep, extract impregnated disks were aseptically placed on the agar surface and after 1 hour of pre incubation diffusion was incubated in an inverted position at 37$^o$C for 18-24 h. Antibacterial activity was determined by measuring the diameter of zones of growth inhibition surrounding the disks. Ciprofloxacin (5 µg) (Oxoid England) and blank filter paper (disk impregnated with methanol only) were used as controls. All experiments were performed in duplicates and the average zones of inhibition in milliliter recorded.

**Determination of Minimum Inhibitory Concentration (MIC) of extract on test bacteria**

The MIC was carried out by agar-dilution method. A stock solution of 250mg/mL was prepared and serially diluted to give six concentrations by graded dilution method in addition to the stock concentrations (250, 125, 62.5, 31.25, 15.63 and 7.81 µg/mL). From the six concentrations, 1 mL each was introduced into six corresponding 20 mL melted and cooled Mueller Hinton agar, mixed and poured into corresponding sterile petri-dishes to give six different concentrations (12.5, 6.25, 3.13, 1.56, 0.78 and 0.39mg/L) of the plant extracts. After the agar had set, the clinical bacteria cell suspensions with optical density equivalent to 0.5 McFarland standards were inoculated onto the six agar plates by surface spreading using sterile swab sticks. The inoculated plates were then incubated at 37$^o$C for 18-24 hours and observed for growth. The lowest concentration of extract that prevented the bacterial growth was recorded as the MIC.

**Determination of Minimum Bactericidal Concentration (MBC)**

The minimum bactericidal concentration (MBC) was determine by sub-culturing from the plates with concentrations equals to the MIC and above on fresh Mueller Hinton broth. The tubes were incubated for 18- 24 hours at 37$^0$C. After incubation the individual tubes were then streaked on fresh Mueller Hinton agar plates and incubated for another 18 - 24 hours at 37$^0$C to detect visible colonies on the agar. The lowest concentration of the extract associated with no bacterial growth was recorded.

**Phytochemical screening of the extracts**

**Preliminary Phytochemical screening of the extracts**

Ten grammes of each of the dried crude extract was weighed and screened for the presence of different classes of secondary metabolites including flavonoids, alkaloids, tannins, saponins, phenols, using previously described standard procedures (Evan, 2009).

**Gas Chromatography-Mass Spectrometry (GC – MS) screening of the extracts**

The screening was carried out using Hewlett-Package 5890 Gas Chromatograph equipped and coupled to VG Analytical 70-250S Mass Spectrometer with a fused silica capillary CP-Sil 5CB column (30m x 0.25 mm ID x 1µm of capillary column). Using Helium as carrier gas at flow rate of 1mL/min, the oven was set to an initial temperature of 90$^o$C, and maintained at this temperature for 2min and then the temperature was heated at 10$^o$C/min to 270$^o$C and finally held isothermally for 15 min. The ionization voltage used was 70eV while a scan of 0.6 s was applied covering a mass range from 36 to 600 amu. The major constituents were identified by matching their MS and retention index data with those of the standards by using computer searches on a NIST version 2.1 MS Library (Mamza *et al*., 2012).

**RESULTS**

**Antibacterial Susceptibility studies**

The antibiotic susceptibility profile of the selected clinical bacteria against the five standard antibiotic disks is shown in Table 1. *E. coli*, *K oxytoca*, *K . pneumoniae*, *S. typhi* and *C. freundii* showed resistance to amoxicillin-clavulanic acid but showed intermediate resistance to cefuroxime. All the bacteria were susceptible to ofloxacin except *Staphylococcus aureus* that showed resistance. The activities of methanolic extracts of *N. lotus* and *S. mombin* impregnated of disks against the isolates are shown in Table 2 in comparison with standard antibiotic disk impregnated with ciprofloxacin (5µg). Highest zones of growth inhibition against all the bacteria were observed against *N. lotus* extracts with *E. coli* having the highest (35mm at

12.5 $mgmL^{-1}$). *S. mombin* extract showed moderate antibacterial activity against all the tested bacteria but showed high inhibitory effect against *C. freundii* and *S. typhi*. Ciprofloxacin, the positive control produced significant zones of growth inhibition against all the test clinical bacteria. The MIC assay for the extracts were found between 3.13 – >12.5$mgmL^{-1}$. The lowest MIC value was 3.13$mgmL^{-1}$against *E.coli, C. freundii* and *S. typhi* for *N. lotus* extract (Table 3) while the MIC values for *S. mombin* was ≥12.5 $mgmL^{-1}$ for all the isolates.

**Phytochemical screening**

The quantitative analysis of *N. lotus* and *S. mombin* extracts is presented in Table 4. The preliminary phytochemical screening revealed the presence of flavonoids, alkaloids, tannins, phenol and saponins in both plants.

**GC-MS Analysis**

The GC-MS analysis revealed the presence of 21 compounds from the extract of *N .lotus* while 25 compounds were obtained from *S. mombin.* The major components present in both plants were benzoic acid derivatives. Other notable bioactive components observed included Sulfurous acid, 2-propyl tridecyl ester (RT: 42.70), Squalene (RT 44. 38), Octadecanoic acid, methyl ester (37.10), 9,12,15- octadecatrienoic acid, methyl ester (Z,Z,Z)- (RT 36.51), 9,12- octadecadienoic acid, methyl ester (RT 36.35), Dibutyl phthalate (RT:33.16), Pentadecanoic acid, 14-methyl-, methyl ester (RT: 32.18) along with other minor constituents were also present (Tables 5 and 6). The GC-MS chromatograms for both plants (Figures 1 and 2) shows the peak separation of the bioactive components.

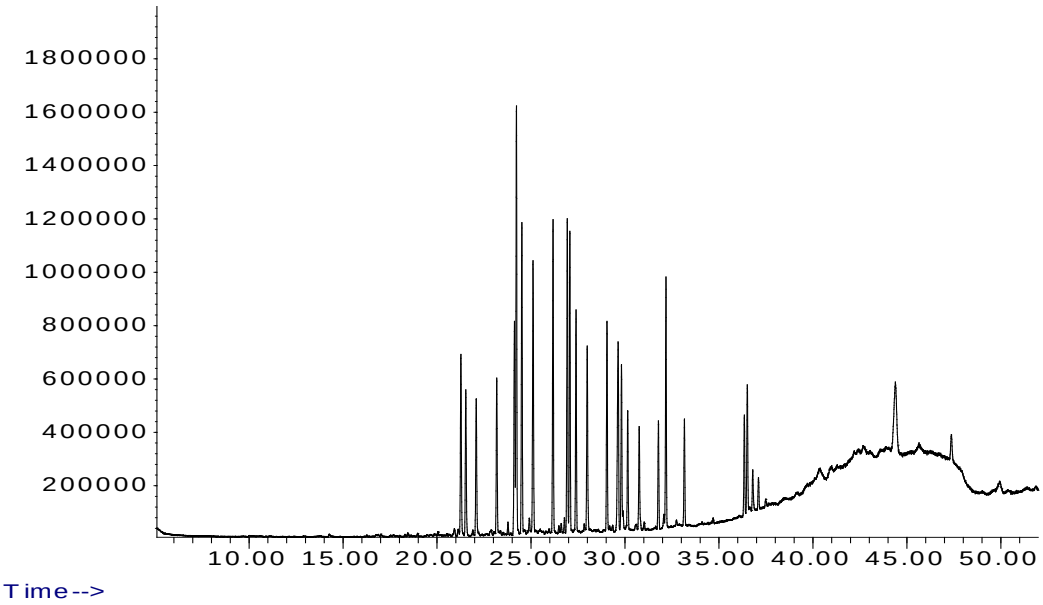


**Figure 1**: Gas Chromatographic-Mass Spectrometric Analysis of *N. lotus* extract

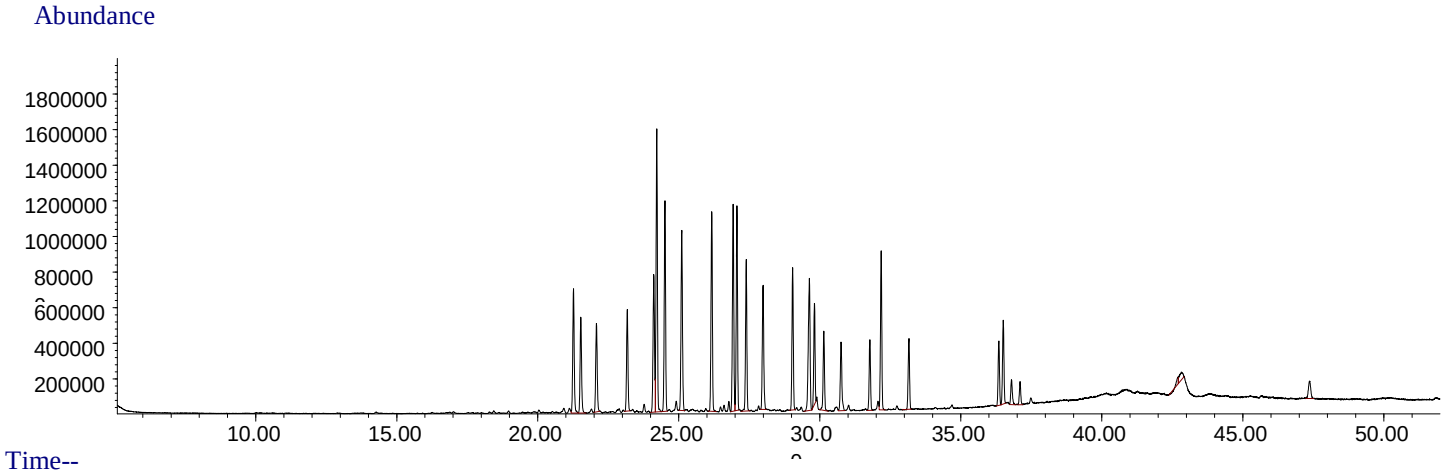


**Figure 2**: Gas Chromatographic-Mass Spectrometric Analysis of *S. mombin* extract

**DISCUSSION**

In the present study, *N. lotus* and *S. mombin* methanol extracts were investigated for the antimicrobial potentials against clinical isolates. Both plants displayed good antibacterial activities on the tested bacterial strains as evidenced in the zones of inhibition observed for both plants (Table 2). There were differences in spectrum of activity for both plants suggesting that they contain different active principles at varying proportions. The phytochemical analysis of *N. lotus* and *S. mombin* (Table 4) revealed the presence of tannins, saponins, alkaloids, flavonoids and phenols at varying proportions. Similar findings have also been previously reported by some authors (Ukwu 2004; Akinjogunola *et al*., 2009). Several authors have previously reported that most of these secondary metabolites possess antimicrobial properties and therefore it is possible that the antimicrobial activities observed in this study may be due to the presence of one or more of these secondary metabolites present in their extracts (Sofowora, 1986; Stray, 1998; Okwu, 2004; Akinjogunola *et al*., 2009). From the analysis carried out in this study, flavonoids, phenols and alkaloids were found to be highly concentrated in *N. lotus* extract than in *S. mombin*, this may contribute to the lower MIC values obtained for *N. lotus* as compared to *S. mombin*. *N. lotus* showed better activity on all the tested clinical bacterial isolates in this study with MIC range of 3.13 – >12.5mg/ml. Of all the bacteria investigated in this study, *E. coli* and *C. freundii* were more sensitive to *N. lotus* extracts while *S. typhi*, *E. aerogenes*, *S. aureus K. oxytoca* and *K. pnuemoniae* were moderately susceptible. On the other hand, *C. freundii*, *S. typhi* and *E. aerogenes* among the other clinical bacteria, showed the highest senitivities to *S. mombin* extract. This result is in agreement with previous reports on the antimicrobial activities of methanol extract of *N. lotus* (Akinjogunola *et al*. 2009; Saadabi and Moglad, 2011) as well as aqueous and organic extracts of *S. mombin* (Aromolaran and Badejo, 2014). Sensitivity of the enteric bacteria such as *S. typhi* and *E. coli* to these extracts justifies their traditional use as curative agents for abdominal diseases and for pregnant women (Elegami *et al*., 2003). Both plants in this study have demonstrated antimicrobial activities against both Gram negative and Gram positive bacteria which is an indication of their broad-spectrum of activities. The observed high MBC for *S. mombin* against *S. aureus* (>12.5 mg/ml), *K. pneumoniae* and *E. coli* are not appreciable considering the prevalence of these bacteria in human infections.

GC-MS analysis of the methanol extracts of *N. lotus* and *S. mombin* investigated in this study revealed the presence of 21 organic metabolites for *N. lotus* and 25 for *S. mombin*,. Benzoic acid derivatives which are known for their good antimicrobial activities (Drãcea *et al*., 2008) are predominant among the constituents of bioactive compounds from the methanol extracts for both plants investigated in this study. Other identified compounds also possess various biological properties that justify the use of both plants for alternative medicine. For instance squalene (RT 44.389) found in *N. lotus* have been shown to have antioxidant property and can effectively inhibit chemical induced skin, colon and lung tumorgenesis in rodents as well as its richness in

rich in vitamin K (Auffray, 2007; Huang *et al.*, 2009). There are other compounds of active bio-components that were also found in extract of *S. mombin* and *N .lotus*. An example is 9, 12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- Fatty acid ester compound that possesses anti-inflammatory, anticancer, antiarthritic and antihistaminic properties (Sermakkani and Thangapandian, 2012) along with many others isolated in this study (Tables 5 and 6 ). Other previous studies with similar compounds from plants have justified the traditional uses of such plant in various countries. The result of the GC-MS analysis of both plants suggests synergy of various bio-components resulting in antimicrobial activities. The presence of these bioactive compounds in plant will be good for the maintenance of human health since they are mostly non-toxic and easily biodegradable. Although the potential antimicrobial activities of both plants have been studied (Akinjogunola *et al*., 2009;Aromolaran and Badejo, 2014), this current work focused on providing additional data on both antimicrobial activities and the presence of bioactive components justifying their traditional use and potential ingredient as good source of alternative medicine and template for drug formulation.

## CONCLUSION

The sensitivity of the selected bacterial strains to the selected plants supports their traditional usage and their potential use as antibacterial agent for novel drug formulation in the treatment of infections. However, further work is needed to isolate the bioactive components of the investigated plants for further studies.

## ACKNOWLEDGEMENTS

The authors appreciated the cooperation of the laboratory technologist and other laboratory staff in the Department of Biosciences and Biotechnology, Babcock University Ogun State, Nigeria on the success of this research work.

**Table 1: Antibiotic susceptibility profiles of standard antibiotics against the test bacteria (clinical bacteria)**

| Test Organisms | Resistance Profile | | | | |
|---|---|---|---|---|---|
| | AUG | CAZ | CRX | GEN | OFL |
| Escherichia coli | R | S | I | I | S |
| Klebsiella oxytoca | R | I | I | I | S |
| Klebsiella pneumonia | R | I | I | S | S |
| Enterobacter aerogenes | I | S | I | S | S |
| Salmonella typhi | R | S | I | R | S |
| Citrobacter freundii | R | S | I | S | S |
| Staphylococcus aureus | S | R | S | S | R |

Key: AUG- Augumentin,CRX- Cefuroxime, CAZ- Ceftazidime, OFL-Ofloxacin, GEN-gentamicin, S-Sensitive, I-Intermediate, R-Resistance

**Table 2**: ***In vitro*** **antimicrobial activities of *N. lotus* and *S. mombin* methanol extracts against clinical bacterial isolates**

| Extracts | Concentrations (mg/mL) | Bacterial isolates zone of inhibition (mm) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | *E. coli* | *K. oxytoca* | *K. pneumoniae* | *E. aerogenes* | *S. typhi* | *C. freundii* | *S. aureus* |
| *N. lotus* | 12.5 | 35 | 23 | 23 | 26 | 22 | 31 | 20 |
| | 6.25 | 24 | 21 | 15 | 16 | 20 | 26 | 10 |
| | 3.13 | 23 | 19 | 8 | 10 | 16 | 23 | 6 |
| *S. mombin* | 12.5 | 15 | 13 | 18 | 14 | 24 | 30 | 15 |
| | 6.25 | 8 | 11 | 12 | 12 | 21 | 26 | 8 |
| | 3.13 | 6 | 6 | 6 | 8 | 12 | 24 | 6 |
| Ciprofloxacin | 5 µg | 40 | 39 | 40 | 31 | 38 | 40 | 43 |
| Methanol | 70% | – | – | – | – | – | – | – |

**Table 3: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *N. lotus* and *S. mombin* extracts on the studied bacterial isolates**

| Test organism | Plant Extracts (mg/mL) | | | |
|---|---|---|---|---|
| | *N. Lotus* | | *S. mombin* | |
| | MIC | MBC | MIC | MBC |
| *Escherichia coli* | 3.13 | 6.25 | >12.50 | >12.50 |
| *Klebsiella oxytoca* | 12.50 | >12.50 | >12.50 | >12.50 |
| *Klebsiella pneumoniae* | 6.25 | 12.50 | 12.50 | >12.50 |
| *Enterobacter aerogenes* | 12.50 | >12.50 | 12.50 | >12.50 |
| *Salmonella typhi* | 3.13 | 6.25 | 6.25 | 12.50 |
| *Citrobacter freundii* | 3.13 | 6.25 | 6.25 | 12.50 |
| *Staphylococcus aureus* | 12.50 | >12.50 | >12.50 | >12.50 |

**Table 4: Qualitative phytochemical screening of *N. lotus* and *S. mombin***

| Extracts | Metabolites | | | | |
|---|---|---|---|---|---|
| | Flavonoids | Tannins | Phenol | Saponins | Alkaloids |
| *N. lotus* | +++ | ++ | +++ | + | +++ |
| *S. mombin* | ++ | ++ | + | +++ | ++ |

Key:

+++ = High concentration, ++ = Moderate concentration, + = Low concentration.

**Table 5: GCMS analysis showing the chemical constituents of *N. lotus***

| Serial No. | Constituents | Retention time (min) | Peak area | Total (%) |
|---|---|---|---|---|
| 1 | Benzene,(1-butylhexyl) | 21.271 | 26109596 | 4.113 |
| 2 | Benzene, (1-propylheptyl) | 21.533 | 19604651 | 3.088 |
| 3 | Benzene, (1- ethyloctyl) | 22.087 | 18601360 | 2.930 |
| 4 | Benzene, (1-pentylhexyl) | 24.121 | 28104752 | 4.427 |
| 5 | Benzene, (1-butylheptyl) | 24.227 | 53076043 | 8.361 |
| 6 | Benzene, (1-propyloctyl) | 24.516 | 39092003 | 6.158 |
| 7 | Benzene, (1-ethylnonyl) | 25.110 | 36175957 | 5.699 |
| 8 | Benzene, (1-methyldecyl) | 26.173 | 38712333 | 6.098 |
| 9 | Benzene, (1-pentylheptyl) | 26.933 | 39405481 | 6.208 |
| 10 | Benzene, (1-butyloctyl) | 27.069 | 37212881 | 5.862 |
| 11 | Benzene, (1-propylnonyl) | 27.399 | 27304626 | 4.301 |
| 12 | Benzene, (1-ethyldecyl) | 27.993 | 24726808 | 3.895 |
| 13 | Benzene,(1-ethylundecyl) | 29.044 | 26433844 | 4.164 |
| 14 | Benzene, (1-hexylheptyl) | 29.639 | 32477253 | 5.116 |
| 15 | Benzene, (1-propyldecyl) | 30.148 | 14842366 | 2.338 |
| 16 | Benzene, (1-ethylundecyl) | 30.759 | 14789533 | 2.330 |
| 17 | Pentadecanoic acid, 14-methyl-, methyl ester | 32.183 | 29611059 | 4.665 |
| 18 | Dibutyl phthalate | 33.165 | 13895589 | 2.189 |
| 19 | 9,12- octadecadienoic acid, methyl ester | 36.352 | 12217901 | 1.925 |
| 20 | 9,12,15- octadecatrienoic acid, methyl ester (Z,Z,Z) | 36.514 | 16840722 | 2.653 |
| 21 | Squalene | 44.389 | 27643281 | 4.355 |

**Table 6: GCMS analysis showing the chemical constituents of *S. mombin***

| Serial No. | Constituents | Retention time (min) | Peak area | Total (%) |
|---|---|---|---|---|
| 1 | Benzene, (1-butylhexyl) | 21.269 | 25,261,165 | 4.096 |
| 2 | Benzene, (1-propylheptyl) | 21.532 | 19,784,131 | 3.208 |
| 3 | Benzene, (1-ethyloctyl) | 22.087 | 18,282,445 | 2.964 |
| 4 | Benzene, (1-methylnonyl) | 23.180 | 19,563,141 | 3.172 |
| 5 | Benzene, (1-pentylhexyl) | 24.118 | 27,824,747 | 4.511 |
| 6 | Benzene, (1-butylheptyl) | 24.221 | 52,605,708 | 8.529 |
| 7 | Benzene, (1-propyloctyl) | 24.513 | 38,985,191 | 6.321 |
| 8 | Benzene, (1-ethylnonyl) | 25.108 | 35,978,733 | 5.833 |
| 9 | Benzene, (1-methyldecyl) | 26.172 | 38,434,322 | 6.231 |
| 10 | Benzene, (1-pentylheptyl) | 26.933 | 39,035,934 | 6.329 |
| 11 | Benzene, (1-butyloctyl) | 27.065 | 36,628,494 | 5.938 |
| 12 | Benzene, (1-propylnonyl) | 27.397 | 27,356,223 | 4.435 |
| 13 | Benzene, (1-ethyldecyl) | 27.992 | 24,571,825 | 3.984 |
| 14 | Benzene, (1-methylundecyl) | 29.039 | 26,320,961 | 4.267 |
| 15 | Benzene, (1-pentyloctyl) | 29.634 | 32,375,042 | 5.249 |
| 16 | Benzene, (1-propyldecyl) | 30.149 | 15,102,935 | 2.449 |
| 17 | Benzene, (1-ethylundecyl) | 30.756 | 14,862,908 | 2.410 |
| 18 | Benzene, (1-methyldodecyl) | 31.780 | 13,518,514 | 2.192 |
| 19 | Hexadecanoic acid, methyl ester | 32.180 | 29,212,694 | 4.736 |
| 20 | Dibutyl phthalate | 33.165 | 13,632,219 | 2.210 |
| 21 | 9,12-Octadecadienoic acid, methyl ester | 36.352 | 12,112,488 | 1.964 |
| 22 | 9,12-Octadecadienoic acid, methyl ester, (Z,Z,Z)- | 36.512 | 16,567,804 | 2.686 |
| 23 | Octadecanoic acid, methyl ester | 37.107 | 4,419,328 | 0.716 |
| 24 | Sulfurous acid, 2-propyl tridecyl ester | 42.703 | 1,088,661 | 0.177 |
| 25 | Octacosane | 42.823 | 3,905,564 | 0.633 |

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