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ISSN 2636–5448 <https://dx.doi.org/10.4314/jopat.v21i2.10>**Screening and Identification of candidate therapeutic drugs from plants for the management of patients infected Bacterial Infection Associated with SARS COV-2****Ibrahim S.I^{1*}, Mukhtar. F.B¹, Abdulkarim. S³, Yarima. M.B³, Ali A⁴, Chifu E⁵, Yamuna K⁶,
Mohammad A.B^{6,7}, Nafisatu K¹, Balogun J.B², Bashir M³, Saifullahi U⁸**¹Department of Biochemistry, Federal University Dutse²Department of Botany, Federal University Dutse³Department of Microbiology/Biotechnology, Federal University Dutse⁴Department of Chemistry, Federal University Dutse⁵Department of Physics, Federal University Dutse⁶College of Medicine and allied Health Sciences, Federal University Dutse⁷North-West Zonal Tuberculosis Reference Laboratory, Department of Medical Microbiology, Aminu Kano Teaching Hospital⁸Department of Pharmacognosy, College of Natural and Pharmaceutical Sciences Bayero University Kano**ABSTRACT**

The emergence of COVID-19 (SARS-COV-2) has resulted in millions of deaths worldwide. Nigeria has recorded over 2000 deaths due to the disease. Development of alternative medicine from plants against SARS-COV-2 can be a potential therapy for treatment of secondary complications. To investigate plants with potential to manage respiratory tract infections. The plants were extracted and screened for phytochemical constituents; their acute and subacute toxicity profile was evaluated. The plant extracts were tested against clinical microbial isolates associated with respiratory tract infection. All the plants extract were found to have acute toxicity dose above 5000 mg per kg body weight. Among the four tested medicinal plants *Guiera senegalensis* was found to have significant zone of inhibition against *Klebsiella pneumonia* and *Moraxella catarrhalis* more than the standard drug (Gentamicin). *Guiera senegalensis* has been used in traditional medicine to treat bacterial infections without any report of toxicity. The preclinical study indicated that *Guiera senegalensis* can be used in the management of respiratory infections associated with symptoms of COVID 19. *Guiera senegalensis* can therefore be used as a potential medicinal plant for management of secondary bacterial infections in Covid19 patients.

Keywords: SARS-COV-2, Covid19, *Guiera senegalensis*, *Klebsiella pneumonia*, *Moraxella catarrhalis***Corresponding author:** Salihu Ibrahim Ismail. Department of Biochemistry, Federal University Dutse. +2348036316108

INTRODUCTION

The emergence of novel coronavirus or COVID-19 (SARS-COV-2) has resulted in millions of confirmed cases and over a million deaths all over the world. There are yet, no single drug to cure SARS-COV-2 infection, and hence the need for in-depth research to uncover candidate therapeutic drugs against SARS-COV-2. Preliminary scientific evidence has revealed that secondary bacterial co-infections in COVID-19 patients lead to complicated difficulties in breathing and increased death tolls.

Respiratory viral infections have been shown to predispose patients to co-infections and these may lead to increased disease severity and mortality. Previous studies have associated most fatalities in the 1918 influenza outbreak due to subsequent bacterial infection, particularly with *Streptococcus pneumoniae* [1]. The 2009 H1N1 influenza pandemic poor outcomes were also associated with bacterial co-infections [2]. Most of the patients who have died in the current coronavirus disease 2019 (COVID-19) pandemic had secondary bacterial infections [3]. Both bacterial and fungal co-infections have been recorded [4]. This necessitates the need to study, identify and manage bacterial pathogens of respiratory tract origin responsible for causing secondary bacterial co-infections in COVID-19 patients. Rapid characterization of co-infection is essential in the management and treatment of the most severe COVID-19 cases which could help to save lives.

The use of alternative medicines from plant-based resources individually and in combination with existing drugs (repurposing/repositioning) to identify candidate therapeutics against SARS-COV-2 will be an interesting area to explore. A vast number of medicinal plants have been recognized as valuable resources of natural antimicrobial compounds as an alternative that can potentially be effective in the treatment of these problematic bacterial infections [6]. According to the World Health Organization (WHO), medicinal plants would be the best source to obtain a variety of drugs [7]. Many plants have been used because of their antimicrobial traits, which are due to phytochemicals synthesized in the secondary metabolism of the plant [8, 9]. Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids, phenolic compounds, and flavonoids, which have been found *in vitro* to have antimicrobial properties [10, 11].

Considering the vast potentiality of plants as sources for antimicrobial drugs, this study aimed to investigate *in vitro* antibacterial activity of extracts from some selected medicinal plants from Jigawa State against the most common respiratory microbial pathogens.

MATERIALS AND METHODS

Sample Collection

Four plant materials were collected from Dutse local government area of Jigawa State, North-west Nigeria, on March 2020 on the basis of traditional medicinal history from the local traditional herbalist and ethno-medicinal use of

plants under this study. The plants were identified and authenticated at the Department of Botany, Federal University, Dutse; Jigawa State, Nigeria.

Preparation of Plant Extracts

The four plants *Calotropis procera*, *Guiera senegalensis*, *Senna occidentalis* and *Hyptis suaveolens* were first washed under running tap water and air-dried in shade at room temperature for a month. Using a home grinder, the plant parts were then ground to fine powder. The weight of the ground powder was taken and the extract from each plant was prepared by using a cold percolation method. This 60 gram of fine powder from each plant was dissolved in 160 ml of absolute methanol at room temperature for three successive days. The supernatant was filtered through Whatman filter paper while the residues were used for a second and third extraction. Each day the dissolved parts were filtered and stored in a glass bottle. After the third extraction, the filtrates were then evaporated under reduced pressure at 50°C using a rotary evaporator to yield the crude extract. The crude extract was collected in a vial for further use.

Preparation of stock solution of extract

Crude extracts 10 mg each were dissolved in 1 mL dimethylsulfoxide (DMSO) and filtered through a sterile syringe filter (0.2 µm pore diameter) to obtain a stock solution (SS; 10 mg/mL). The SS was diluted with the

maintenance medium to obtain a final concentration of 1000 µg/mL (0.1% DMSO).

Microbial Culture

A total of 6 human pathogenic microbial strains were used in the study *Streptococcus pneumonia*, *Klebsiella pneumonia*, *Moraxella catarrhalis*, *E.coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. A series of morphological, physiological, and conventional biochemical tests were performed to identify the selected microorganisms. Antimicrobial susceptibility test was performed for all microbial isolates by modified Kirby Bauer disc diffusion method following the Clinical and Laboratory Standards Institute (CLSI) guideline. Multidrug resistant (MDR) isolates was defined as those isolates that are resistant to three classes of antibiotics [12].

Antimicrobial Assay of Plant Extracts

Antimicrobial assay of extracts of different plants were performed by agar well diffusion method in Mueller Hinton Agar (MHA) plates. The test organisms were inoculated in Nutrient broth and incubated overnight at 37°C to adjust the turbidity to 0.5 McFarland standards giving a final inoculum of 1.5×10^8 CFU/ml. MHA plate were lawn cultured with standardized microbial culture broth. Plant extracts of 50 mg/ml concentration were prepared in Dimethyl Sulfoxide (DMSO). Six wells of 6 mm were bored in the inoculated media with the help of

sterile cork-borer (6 mm). Each well was filled with 50 μ l extracts from different plants: positive control (amikacin 30 mcg and nitrofurantoin 300 mcg) for bacteria and negative/solvent control (DMSO), respectively. It was allowed to diffuse for about 30 minutes at room temperature and incubated for 18-24 hours at 37°C. After incubation, plates were observed for the formation of a clear zone around the well which corresponds to the antimicrobial activity of tested compounds. The zone of inhibition (ZOI) was observed and measured in mm.

Determination of MIC and MBC of the Plant Extracts

The broth microdilution method was used to determine the MIC according to CLSI. Streaks was taken from the two lowest concentrations of the plant extract plates exhibiting invisible growth (from inhibition zone of MIC plates) and subcultures onto sterile Tryptone soya agar (TSA) plates. The plates were incubated at 35 °C for 24 h. then examined for bacterial growth in corresponding to plant extract concentration. MBC was taken as the concentration of plant extract that did not exhibiting any bacterial growth on the freshly inoculated agar plates.

Statistical analysis

Data collected were analysed using SPSS 20.0 to determine t-test and analysis of variance.

RESULTS

A total of six plants extracts were tested by agar well diffusion method in Mueller Hinton Agar (MHA) plates using different strains of bacteria (*Streptococcus pneumonia*, *Klebsiella pneumonia*, *Moraxella catarrhalis*, *E. coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*). Poly herbs show the highest zone of inhibition 25.9% as shown in table 1 and 2 below against the above bacteria, while the lowest MIC was observed with *Guiera senegalensis* 8.08% against above bacteria. *Guiera senegalensis* shows MIC 23.5.0 \pm 1.0 with *E. coli*, while the control (Gentamycin) shows MIC 20 \pm 0.0 against the bacteria (*E. coli*). *Hyptis suaveolens* had MIC of 16.0 \pm 1.1 against *Streptococcus pneumonia* while MIC 06 \pm 0.2 was observed with *Senna occidentalis* against *Moraxella catarrhalis* as shown in table 1 below.

Table 2 below shows that, plant extract of *Guiera senegalensis* shows the highest zone of inhibition of 23.5.0 \pm 1.0 against *E. coli*; while Poly herb had the lowest MIC with *E. coli* compare to *Staphylococcus aureus* (16.0 \pm 2.0) and *Pseudomonas aeruginosa* (7.5 \pm 0.9) respectively. Other plants extract show different MIC against other bacteria like *Staphylococcus aureus* and *Pseudomonas aeruginosa* as shown below.

Table 1: Extract yield, Minimal Inhibitory concentration (MIC) of four leaf extracts against *Streptococcus pneumonia*, *Klebsiella pneumonia* and *Moraxella catarrhalis*

		<i>Streptococcus pneumonia</i>	<i>Klebsiella pneumonia</i>	<i>Moraxella catarrhalis</i>
Plants	% yield	MIC (mg/ml)	MIC (mg/ml)	MIC (mg/ml)
<i>Calotropis procera</i>	21.7	13.4±4.0	7.4±2.2	11.2±5.2
<i>Guiera senegalensis</i>	8.08	13.3±1.5	13.5±1.1	20.0±2.8
<i>Senna occidentalis</i>	15.7	14.0±1.0	10±2.0	06±0.2
<i>Hyptis Suaveolens</i>	13.3	16.0± 1.1	9.0±1.3	11.0±3.0
Poly herb	25.9	8.5±2.1	13.0±2.5	6.0±1.0
Gentamicin	NA	20±0.0	10±0.0	15.0±0.0
Control				

Table 2: Extract yield, Minimal Inhibitory concentration (MIC) of four leaf extracts against *E. coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*

		<i>E. coli</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>
Plants	% yield	MIC (mg/ml)	MIC (mg/ml)	MIC (mg/ml)
<i>Calotropis procera</i>	21.7	12.0±5.5	11.6±6.6	9.0±1.0
<i>Guiera senegalensis</i>	8.08	23.5.0±1.0	18.0±4.0	17.3±3.0
<i>Senna occidentalis</i>	15.7	7.5±2.0	15.1±1.8	10.0±1.0
<i>Hyptis suaveolens</i>	13.3	16.0±1.5	12.0±1.0	12.0±1.0
Poly herb	25.9	6.0±1.0	16.0±2.0	7.5±0.9
Gentamicin	NA	22.0±0.0	25.0±0.0	34.0±0.0
Control				

DISCUSSION

Natural products remains to be a wealthy source for the identification of novel therapeutic agents for the treatment of human diseases. The present study is based on the central premise that when pathogens invade the body to cause diseases, they reduce the immune function of the body [13]. In general, the reduced immune function is further weakened if only simple anti-pathogen treatment is used. Herbal medicine improves immune function by improving the body's resistance to disease, physical conditions and by reducing the side effects of Western medicine [14].

Several studies have been conducted to study the effectiveness of herbal medicine in the treatment of COVID-19 [15]. However, in the present study, we focused only on the recently and most frequently used herbal medicine for treating flu like infections with severe fever and acute respiratory distress, which may be helpful in the management of Covid 19 patient with secondary bacterial infection.

Overwhelming inflammatory responses are attributable to the deaths of patients with infection of SARS-CoV, or MERS-CoV, or COVID-19. Thus, anti-inflammatory agents presumably could reduce the severity and mortality rate [16]. *Guiera senegalensis* a medicinal plant that is widely used in West Africa against many illnesses extract was found to have higher zone of inhibition in the present

study. The plant extract contained phytochemicals such as flavonoids, alkaloids, steroids, tannins and saponins. *Guiera senegalensis* was found to possess anti-asthmatic activity [17]. Which makes it a potential candidate for use against acute respiratory syndrome. In a similar study the extracts of the leaves of *Guiera senegalensis* were studied in vitro against *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli* and *Streptococcus pyogenes*. The study shows that the leaf of *Guiera Senegalensis* possess antibacterial property [18].

CONCLUSION

The four medicinal plants (*Calotropis procera*, *Guiera senegalensis*, *Senna occidentalis*, *Hyptis suaveolens* and Poly herb) discussed here collectively exhibited pleiotropic effects which can potentially provide a multimodal approach against respiratory bacterial infection associated with SARS COVID 19 infection. At present, it is evidently challenging to pool data from published studies due to variation in plant extracts selection and a lack of well-reported standardisation data of the investigated formulations. Still, it is quite clear that there is insufficient evidence of direct antiviral effects specific to the SARS-CoV-2.

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