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Research Article

Methanol Extract of *Combretum dolichopentalum* Exhibits Broad-spectrum Antimicrobial Effect on Nosocomial Organisms

[1]Ujowundu Favour Ntite, [2]Oparaeche Nathan Ndudi, [3]Ujowundu Cosmas Onyekachi, [4]Nwachukwu Ikenna Ndubuisi, [1]Nwobodo Afoma Maria and [1]Enomfon Okokon Udoh

[1]Department of Biochemistry, Madonna University Nigeria, Elele Campus, Rivers' State, Nigeria
[2]Department of Pharmaceutical Chemistry, Madonna University Nigeria, Elele, Rivers State, Nigeria
[3]Department of Biochemistry, Federal University of Technology, Owerri, Nigeria
[4]Department of Microbiology, Federal University of Technology, Owerri, Nigeria

Abstract

Background and Objective: The emergence of resistant strains of bacteria and fungi has resulted in an increase in nosocomial infections which has become a worldwide problem in clinical medicine. This study investigated an alternative source of potent antimicrobial agents. It provided evidence that embedded in *Combretum dolichopentalum* are phyto-active constituents capable of exhibiting broad-spectrum antimicrobial effect on Gram-positive and Gram-negative organisms, the two major disease-causing bacteria groups not excluding fungi. **Materials and Methods:** Isolates of *Escherichia coli*, *Streptococcus pneumonia*, *Staphylococcus aureaus*, *Klebsiella pneumoniae*, *Pseudomonas aeroginosa* and *Candida albicans* were tested against methanol extract of *C. dolichopentalum* using 2, 3, 5-triphenyltetrazolium chloride (TTC) to assay total dehydrogenase activity (DHA) which indicates the viability of microorganisms in the absence of antibiotics. **Results:** Methanol extract of *C. dolichopentalum* dose dependently inhibited 50 and 80% of the isolates at concentrations of 503.54 and 1801.41 μg mL^{-1} for *E. coli*, 253.12 and 1077.24 μg mL^{-1} for *S. aureaus*, 732.5954 and 3047.6054 μg mL^{-1} for *S. pneumonia*, 681.8154 and 2234.3454 μg mL^{-1} for *K. pneumoniae*, 111.8654 and 554.9954 μg mL^{-1} for *P. aeroginosa* and 171.88 and 454.33 μg mL^{-1} for *C. albicans*, respectively. These activities portray a promising phyto-antimicrobics derived from crude extract of *C. dolichopentalum*, not only for the potency or for the presence of a wide array of bioactive phytochemicals but also for the absence of significant side effects when administered. **Conclusion:** The antimicrobial potency recorded indicates that *C. dolichopentalum* could be the pharmacological tool needed to unlock the intrinsic gateway to resolving human health challenges.

Key words: Asymptomatic, nosocomial, antimicrobial, *C. dolichopentalum*

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Corresponding Author: Ujowundu Favour Ntite, Department of Biochemistry, Madonna University Nigeria, Elele Campus, Rivers State, Nigeria

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

A nosocomial infection is contracted as a result of an infection or toxin that exists in locations, such as a hospital[1]. Nosocomial infections are caused by bacteria, fungi and viruses. *Streptococcus pneumonia*, a Gram-positive human pathogenic bacterium is the subject of many humoral immunity studies[2,3]. It is also regarded as a major cause of pneumonia which resides a symptomatically in healthy carriers colonizing the respiratory tract, nasal cavity and sinuses. However, in vulnerable persons with weaker immune systems, such as the elderly and young children, the bacterium may become pathogenic and spread to other organs to cause diseases[4] such as, acute sinusitis, bronchitis, brain abscess, cellulitis, conjunctivitis, endocarditis, meningitis, otitis, osteomyelitis, pericarditis, peritonitis, rhinitis, septic arthritis and sepsis[5-7]. *Klebsiella pneumonia* is a Gram-negative, bacteria which infects mostly individuals with weakened immune system. For patients with an invasive device in their bodies, contamination of the device becomes a risk. Klebsiella organisms are often resistant to multiple antibiotics. Current evidence implicates plasmids as the primary source of the resistance genes[8-9].

About 20-30% of human population is estimated to be long-term carriers of Gram-positive *Staphylococcus aureus*[10] as part of the normal microbiota present in the gut mucosa, on skins, in the nostrils[11], in the upper respiratory tract[12] and in the lower reproductive tract of women[13]. Infections caused by *S. aureus* are generally associated with breakages in the skin or mucosal membranes due to surgery, injury or use of intravascular devices. On entering the bloodstream, bacteria can infect various organs[14].

Escherichia coli, a Gram-negative bacillus, causes a vast majority of neonatal meningitis[15-16]. *Escherichia coli* intra-abdominal infections often result from a perforated viscus (e.g., appendix, diverticulum)[17], microaspiration of upper airway secretions of normal microbiota constituting this organism in severely ill patients. Other miscellaneous *E. coli* infections includes, endocarditis, endophthalmitis, osteomyelitis, suppurative sinusitis, septic arthritis, skin and soft-tissue infections and thyroiditis (especially in patients with diabetes)[18]. Among the most common causes of foodborne diseases is shiga toxin-producing *E. coli* (STEC)[19], responsible for several GI illnesses, including nonbloody and bloody diarrhea. Sufferers of these diseases, may be affected by neurologic hemolytic-uremic syndrome (HUS)[20].

Another Gram-negative, bacterial responsible for nosocomial infections is *Pseudomonas aeruginosa*[21]. Often *P. aeruginosa* colonizes immunocompromised patients, suffering from AIDS or cancer[22]. The potency of this pathogen is such that firstly, it attacks up to two thirds of the critically-ill hospitalized patients, which usually portends more invasive diseases. Secondly, *P. aeruginosa*, carries a 40-60% mortality rate at most medical centers. Thirdly, it aggravates 90% of cystic fibrosis deaths and lastly, it is connected to the worst visual diseases[23-24].

Finally, overgrowth of *C. albicans*, usually in immunocompromised individuals[25], leads to diseases such as invasive candidiasis, oropharyngeal candidiasis and vulvovaginal candidiasis[26]. Interestingly, as the 4th leading cause for nosocomial infections in patients 'bloodstreams, this could result in an extremely life-threatening, systemic infection in hospital patients with a mortality[27] rate of 30%.

Efforts required to prevent and control nosocomial infections includes: Adopting antibiotic control policy and monitoring antimicrobial use and its resistance[28]. However, resistance to antimicrobial agents has become an increasingly important and pressing global problem. Substantial investment and research in the field of anti-infectives are now desperately needed if a public health crisis is to be averted.

Studies have shown that plants synthesize lower molecular weight organic compounds which possess various biological and therapeutic activities and are used as source of potent drug in folklore medicine to treat various ailments[29-31]. The ethanol extract of *C. dolichopentalum* is used by the people of Umunama in Ezinihitte Mbaise LGA and around Ogwa both in Imo State of Nigeria[32] for treating several ailments like gastrointestinal disorders. Both the aqueous and the ethanol extracts of the leaves of *C. dolichopentalum* possess antioxidant activities[33]. This study determined the antimicrobial effect of methanol extract of *C. dolichopentalum* on some microbial isolates.

MATERIALS AND METHODS

This study was carried out at Obinze in Owerri West Local Government Area of Imo state, Nigeria from January-April, 2017.

Preparation of plant material: Fresh leaves of *C. dolichopentalum* were harvested from a farm at Obinze in Owerri West Local Government Area of Imo state of Nigeria. The location GPS coordinates were: N5°23'41.1' and E 6°57' 14.0' with 60 m elevation. The identification of the plant was done by plant taxonomist: Dr. F.N. Mbagwu of the Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria. A specimen of the plant sample was deposited at Imo State University Herbarium with a voucher IMSUH12. Fresh leaves of *C. dolichopentalum* were removed from

their stems, washed with clean water and allowed to dry at 28±2°C room temperature. Finally, dried leave samples were pulverized with the aid of electric blender and stored in an airtight container placed in a desiccator for 3 days. Afterwards, the dried sample was extracted as described by Bohm and Koupai-Abyazani[34]. Briefly; dried sample of 112 g were extracted repeatedly with 1125 mL of 80% aqueous methanol at room temperature. Whatman No. 1 filter paper was used to filter the solution obtained. The combined filtrate was concentrated at mild temperature using water bath. The precipitate obtained was tested for presence of flavonoids using standard methods[35].

Isolation of organism: Clinical bacterial isolate of *S. aureus* (ATCC 25923), *P. aeruginosa* (ATCC 27853), *K. pneumonia* (ATCC 34089), *C. albicans* (ATCC 22018) obtained from the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria were used for the study. *Escherichia coli* were isolated from infected stool and *Streptococcus* spp. was isolated from high vaginal swab. Microorganisms were sub-cultured in nutrient broth and characterized by the use of standard microbiological and biochemical methods as described by Holt *et al.*[36]. The bacterial isolates were grown to mid exponential phase in nutrient broth on a rotatory incubator (150 rpm) at room temperature (28±2°C). Afterwards, the cells were harvested by centrifugation at 3000 g for 10 min and washed thrice in distilled water. The washed cells were re-suspended in distilled water and turbidity adjusted to an optical density of 0.1 at 540 nm. An aliquot of 0.1 mL of the cell suspension was used as inoculum in the dehydrogenase assay.

Experimental design: Total dehydrogenase assay method as described by Alisi *et al.*[37] was employed to determine the antimicrobial activity of the extract. Briefly, total dehydrogenase activity was assayed using 2, 3, 5-triphenyltetrazolium chloride (TTC) (BDH England) as the artificial electron acceptor, which was reduced to the red-colored triphenyl-formazan (TPF). The assay was carried out in a 2 mL volumes of nutrient broth-TTC medium supplemented with varying concentrations (0-3000 µg mL^{-1}) of extract in separate 20 mL screw-capped test tubes. Portions (0.1 mL) of the bacterial suspensions were inoculated into triplicate glass tubes containing 0.5 mL of x4 strength nutrient broth medium amended with methanol extract of *C. dolichopentatum* (MECD), phosphate-buffer (pH 6.8) and pre-incubated on a rotary incubator (150 rpm) at room temperature (28±2°C) for 30 min. Thereafter, 0.1 mL of 0.1% (w/v) TTC in deionised distilled water was added to each tube to obtain final extract concentrations of 0- 3000 µg mL^{-1} in different test tubes. The controls consisted of the isolates and the media without extract. The reaction mixtures were further incubated statically at room temperature (28 ±2°C) for 24 h. The TPF produced were extracted in 4 mL of butanol and determined colorimetrically at 500 nm.

Calculation:

$$\text{Inhibition of DHA activity}(\%) = 100 - \frac{\text{Absorbance of test}}{\text{Absorbance of control}} \times 100 \quad (1)$$
$$= 100 - \text{DHA of control } (\%)$$

Inhibition of dehydrogenase activity of the isolates by the methanol extract of *C. dolichopentalum* (MECD) was calculated relative to the control. The percentage inhibitions for organisms were plotted against the concentrations of the extracts using the Table 2, D curve V 5.01 system software. The toxicity threshold concentrations (IC_5, IC_{10}, IC_{20}, IC_{30}, $1C_{50}$, $1C_{80}$ and $1C_{100}$) were then evaluated from the dose response plots. The total inhibitory concentrations ($1C_{100}$) values which were non-determinable from the simple inhibition plots were subjected to evaluation using a log transformation of inhibition (%) plots. Note that: Log Inhibition (%) = 2 = $1C_{100}$.

Data/statistical analysis: The plant extract total dehydrogenase inhibition data (mean values from triplicate determinations) were fitted into kinetic equation-logistic-dose-response model and sigmoid abcd model using Levenberg-Marquardt algorithm (Table curve 2D SYSTAT USA)[38]. Percentage inhibition of dehydrogenase activity in pathogens by methanol extract of *C. dolichopentalum*, was calculated relative to their controls as shown in Eq. 1. Furthermore, calculated percentage inhibition data were fitted into the logistic dose response model (Eq. 1) by plotting inhibition (y) against extract or standard concentration (x).

RESULTS

The result presented in Fig. 1 shows that MECD dose dependently inhibited total dehydrogenase activity in *E. coli* following the logistic dose response curve. At IC_{100} *E. coli* recorded the highest threshold inhibitory concentration as shown in the Table 1.

At IC less than that against *E. coli* the extract dose dependently inhibited total dehydrogenase activity in *S. aureus* following the logistic dose response curve (Fig. 2). Threshold inhibitory concentrations are as seen in the Table 1. The result presented in Fig. 3 reveals that the extract

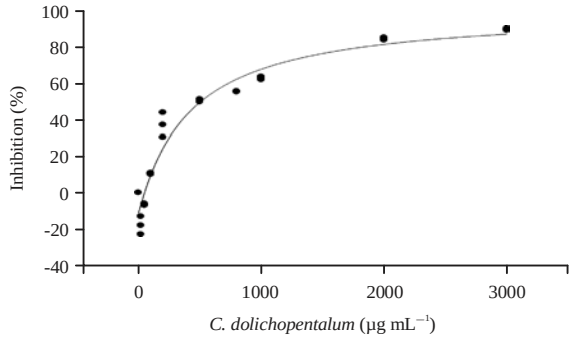

Fig. 1: Effect of methanol extract of *C. dolichopentalum* (MECD) on inhibition of total dehydrogenase activity in *E. coli*

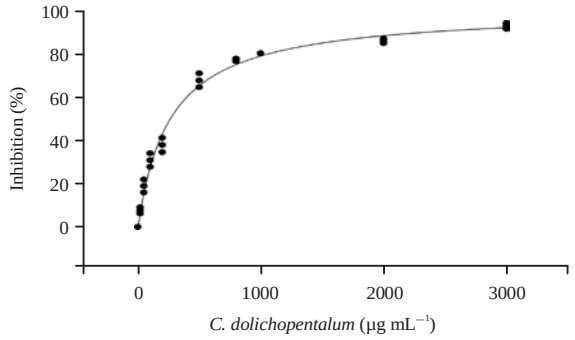

Fig. 2: Inhibition of total dehydrogenase activity in *S. aureus* by MECD

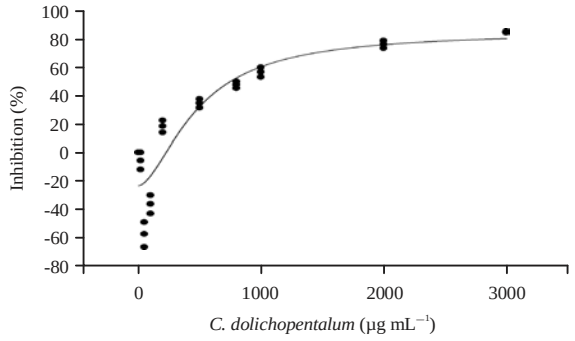

Fig. 3: Effect of MECD in *Streptococcus* spp.

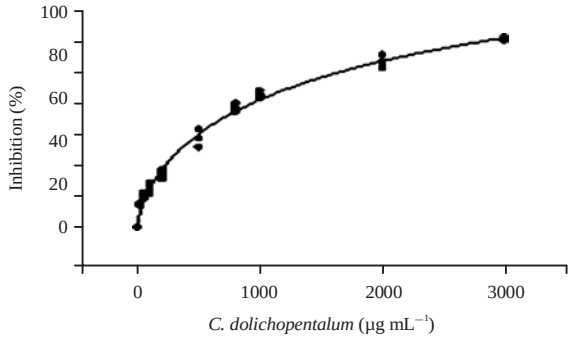

Fig. 4: Inhibition of total dehydrogenase activity in *K. pneumonia* by MECD

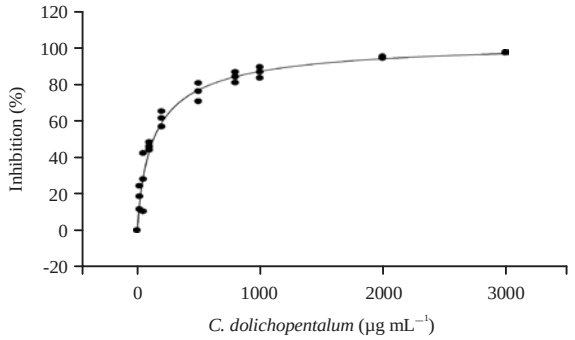

Fig. 5: MECD dose dependently inhibited total dehydrogenase activity in *P. aeruginosa*

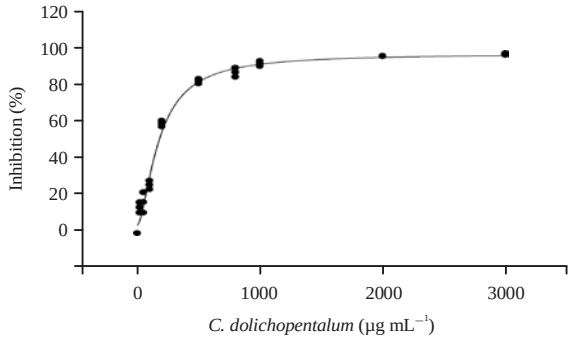

Fig. 6: Inhibitory concentrations of the methanol extract of *C. dolichopentalum* leaves on *C. albicans*

dose dependently inhibited total dehydrogenase activity of *S. pneumonia* with an IC higher than that recorded by *S. aureaus*. However, threshold inhibitory concentrations at IC_{100} were non-determinable as displayed in Table 1.

Also, the effect of MECD on *K. pneumoniae* shown in Fig. 4 indicates that MECD dose dependently inhibited total dehydrogenase activity in *K. pneumoniae* following the logistic dose response curve in a manner less than that observed in *S. aureaus*. The threshold inhibitory concentrations are as indicated in Table 1.

Furthermore, the result shown in Fig. 5 reveals that the extract dose dependently inhibited total dehydrogenase activity in *P. aeruginosa* following the logistic dose response curve at IC_5, IC_{10}, IC_{20}, IC_{30}, IC_{50} and IC_{100} less than that seen in other bacterial in this study. Threshold inhibitory concentrations are as observed in Table 1.

Finally, the results presented in Fig. 6 shows that the extract dose dependently inhibited total dehydrogenase activity in *C. albicans* following the logistic dose response curve at IC. At IC_{80} *C. albicans* recorded an inhibitory

Table 1: Inhibitory concentrations of the methanol extract of *C. dolichopentalum* leaves against the total dehydrogenase activity (DHA) of some microbial isolates

	Inhibitory concentration against isolates						
	MECD (μg mL^{-1})						
Species name	IC_5	IC_{10}	IC_{20}	IC_{30}	IC_{50}	IC_{80}	IC_{100}
Escherichia coli	73.10	101.21	166.13	247.58	503.54	1801.41	8735.80
Staphylococcus aureaus	10.98	25.13	59.56	104.76	253.12	1077.24	5835.89
Streptococcus spp.	255.08	293.33	374.01	466.31	732.59	3047.60	ND
Klebsiela pneumonia	5.70	32.97	116.74	244.35	681.81	2234.34	4680.29
Pseudomonas aeruginosa	2.56	6.78	19.33	38.56	111.86	554.99	4485.49
Candida albicans	10.97	30.96	63.55	94.96	171.88	454.33	ND

ND: Non-determinable

concentration less than that of the other organisms but were non-determinable at IC_{100} as revealed in Table 1.

DISCUSSION

The inhibitory effect of methanol extract of *C. dolichopentalum* on *E. coli*, *S. pneumonia*, *S. aureaus*, *K. pneumoniae*, *P. aeroginosa* and *C. albicans* were thus evaluated and our result (Fig. 1-6) shows that MECD exhibited a broad-spectrum antimicrobial potential, as it acts on the two major disease-causing bacteria groups, Gram-positive and Gram-negative[39]. The antimicrobial activities of *C. dolichopentalum* is not only limited to bacteria, it has also shown potentials of inhibiting the growth of fungi (*C. albicans*) dose dependently.

Broad-spectrum antibiotics are used: When the causative organism is unknown but procrastination of treatment would amount to aggravating infection or bacteria disseminating to other parts of the body, secondly for bacteria resistant to narrow-spectrum antibiotics, thirdly, in the case of manifold infections, where there are various species of bacteria causing illness, thus requiring either a broad-spectrum anti-biotic or associated anti-biotic therapy and finally, for protection in order to prevent the occurrence of bacterial infections. This can occur before surgeries, to stop infection during the operation or for patients with immunocompromised status who are at high-risk for dangerous bacterial infections[40].

Results shows that MECD inhibited the Gram-positive bacteria *S. aureaus* better than *S. pneumonia* as it recorded a greater inhibition of the organism at lower dose concentration compared to *S. pneumonia*. Although both *S. aureaus* and *S. pneumonia* are Gram-positive bacteria, however, *S. aureaus* is a catalase-positive strain while *S. pneumonia* is a catalase-negative strain. Catalase-negative strains have been shown to grow more rapidly than the catalase-positive strains under aerobic or anaerobic conditions in a glucose-containing complex medium, thus the ability to survive better[41].

Carbon dioxide dependent strains of *S. aureaus* have decreased metabolism and a defective electron transfer system and are auxotrophic for substrates such as haemin, menadione, thiamine or thymidine[42]. They also have an intrinsic resistance to aminoglycoside antibiotics such as gentamicin and are most frequently identified in patients with chronic or persistent infections[43]. Multi-resistance to antibiotics has most often been associated with meticillin resistant strains[43] due to its virulence factors[44]. However, this study suggested that *C. dolichopentalum* can be employed to effectively inhibit the activities of the *S. aureaus* infections in humans irrespective of its catalase ability to enzymatically degrade phenolic compounds which occur naturally in plants[45].

Compared to *E. coli* and *K. pneumonia*, *P. aeroginosa* achieved better inhibitory capacity. The outer membrane surrounding the cell wall of *E. coli* provides a barrier to certain antibiotics such that *E. coli* is not damaged by penicillin[46], thus the relative higher inhibitory concentrations needed to inhibit *E. coli*. The mortality rate of Klebsiella bacteria infection can be nearly 100% for people with alcoholism and bacteraemia[47], thus making it deadly and calls for appropriate attention. Current evidence implicates plasmids as the primary source of the resistance genes of Klebsiella to multiple antibiotics[8,9].

The *C. dolichopentalum* has shown the *in vivo* and *in vitro* ability to scavenge reactive oxygen species[32,33], thus was able to inhibit the activities of *P. aeruginosa*. *Pseudomonas aeruginosa* uses the virulence factor exotoxin A to inactivate eukaryotic elongation factor 2 by means of ADP-ribosylation in the host cell, just as the diphtheria toxin does. Without elongation factor 2, eukaryotic cells cannot synthesize proteins and as a result necrotise. The release of intracellular contents initiates an immunologic response in immunocompetent patients. In addition, *P. aeruginosa* uses an exoenzyme, ExoU, which degrades the plasma membrane of eukaryotic cells, leading to lysis. Increasingly, it is becoming

recognized that the iron-acquiring siderophore, pyoverdine, also functions as a toxin by removing iron from mitochondria, inflicting damage on this organelle[48,49].

One of the most perturbing characteristics of *P. aeruginosa* is its low antibiotic vulnerability; therefore, research for the discovery of new antibiotics and drugs against *P. aeroginosa* is very much needed. *C. dolichopentalum* could be the source of that antibiotic.

Furthermore, filamentous cells have in common many similarities with yeast cells. Both cell types seem to play a definite, characteristics role in the survival and pathogenicity of *C. albicans*. Yeast cells seem to be better suited for the dispersal in the bloodstream while hyphal cells have been proposed as a virulence factor[50-52]. When *C. albicans* cells are grown in a medium that imitates the physiological environment of a human host, they proliferate as filamentous cells (both true hyphae and pseudohyphae). The *C. albicans* can also form Chlamydospores, which may play a role in surviving harsh environments as they are most often formed under unfavorable conditions[53]. Exposure of *C. albicans* to *C. dolichopentalum* has shown promising antifungal potentials against *C. albicans*. At a relatively lower dose, the extract was able to inhibit the activities of *C. albicans* effectively. This suggests that *C. dolichopentalum* could be a good source of antifungal agent.

Combretum dolichopentalum exhibits bioactive contents[33] which may serve as replacement to synthetic agents[33,54]. This is arisen from increasing usage limitations of synthetic therapeutic agents due to side effects and resistances. Saponin has been shown to kill or incapacitate living organisms such as fish[55] probably by complexing with cholesterol to form pores in cell membrane bilayers, leading to cell lysis[56] and thus *C. dolichopentalum* could possibly have such effect on nosocomial organisms as it contains saponins which are also responsible for many other important activities-anthelmintic, anti-bacterial, anti-oxidant, anti-malarial, immunomodulatory and Molluscidal[57].

In addition, the flavonoids content of *C. dolichopentalum*[33], at cellular levels, may exert a variety of biological effects[58] as anti-oxidants, anti-inflammatory and anti-cancer agents as well as its alkaloid content which may also possess anti-proliferative potencies, anti-tumour activity and antimicrobial effect in cancer cells due to the presence of octadecenamide[59].

CONCLUSION

Furthermore, due to the resistance of nosocomial organisms to antimicrobial agents, the need to seek or employ an alternative source of antimicrobials to address this pressing global problem led to the exploitation of *C. dolichopentalum.* Derived from the crude methanol extract of *C. dolichopentalum* is a promising phyto-antimicrobics due to the presence of bioactive therapeutic agents. If isolated, characterized and purified could resolve untold health challenges as indicated in this study.

SIGNIFICANCE STATEMENT

This study discovered an alternative source of potent antimicrobial agents that can be beneficial for exhibiting broad-spectrum antimicrobial effect on nosocomial organisms. This study will help the researchers to uncover the critical areas of the therapeutic potentials of this plant that many researchers were not able to explore. Thus a new theory, on why for centuries, the indigenous people of Imo state have administered *C. dolichopentalum* to women after parturition may be arrived at.

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