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ASSESSMENT OF THE ANTIMICROBIAL ACTIVITY OF *Eucalyptus camaldulensis* PLANT EXTRACTS

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ABSTRACT

The Eucalyptus camaldulensis plant is extensively used in traditional medicines for treating many diseases. This has prompted the aim of this study to investigate the *in vitro* antimicrobial activity of the plant to validate its local usage. The leaves and stem barks of *E. camaldulensis* were subjected to ethanolic extraction via the maceration technique. Standard methods were adopted for the screening of various bioactive phytochemical constituents of the extracts of *E. camaldulensis.* Varied concentrations between 20 to 100 mg/mL were applied for the antimicrobial susceptibility test against purified microbial isolates (*S. aureus, Salmonella typhi, P. aeruginosa, and C. albicans*) via the agar wells technique. Lower standards of range 1.56 to 25 mg/mL were adopted for the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) determination. Findings from this study revealed high antimicrobial activity, particularly against bacterial test organisms. The lowest extract concentration (20 mg/mL) produced an 18 mm zone of inhibition against the bacterial test organism while 40 mm was the highest produced at 100 mg/mL. The lowest MIC detected at 6.25 mg/mL further accentuated the inhibitory capability of the *E. camaldulensis* plant extracts against the test organisms, though no variation in MIC activity between the leaf and stem bark extracts. This research supported the antimicrobial potency of *E. camaldulensis* extracts which was attributed to its rich sources of phytochemicals.

Keywords: *E. camaldulensis,* Minimum Inhibitory Concentration, Extracts, Antimicrobial Activity, Traditional Medicine, Minimum Bactericidal Concentration

INTRODUCTION

More than 50% of present-day therapeutic drugs originate from natural products, consequently, these natural products have played a vital role in the production of new drugs in today's pharmaceutical industry (Jeyachandran and Mahesh, 2007). It is widely reported that herbal and medicinal plant use dates to the stretched ancient periods, thus researchers per its historical therapeutic benefits now quest for substitute antimicrobial medicines mostly of plant origin (Najah, 2014; Lin *et al.,* 2015).

The herbal and medicinal plant use as an old-fashioned healthcare approach is common in 80% of the global population including Asia, Africa and Latin America, though reported as having fewer downsides (Lin *et al.,* 2015; Motamedi *et al.,* 2014). A typical natural source of drugs and antimicrobial agents is plant and plant products. The plants of medicinal value have long usage in treating different types of diseases globally before the discovery of modern drugs; and are recognized to contain potent phyto-substances (Pathania *et al.,* 2020). Sabo and Knezevic (2019) also attested that medicinal plants have hugely impacted improving the healthcare system and signify a substantial source of innovative antimicrobials applied in tackling

drug-resistant infectious microbes. Consequently, these notably necessitate the quest for novel potent antimicrobials, especially from plant sources, and the *Eucalyptus camaldulensis* plant is one of the ancient plants mostly associated with medicinal properties in traditional settings.

The plant *E. camaldulensis* is of vital ethnomedicinal value and belongs to the family Myrtaceae, with the genus being native to Tasmania and Australia and having above 800 to nearly 900 species (Coppen, 2002; Pereira *et al.,* 2014). The *Eucalyptus camaldulensis* is also called by several other common names such as red gum, long beak eucalyptus, and river gum, and is notably referred to as river red gum, red gum eucalyptus and Murray red gum due to its ability to produce a significant amount of red gum (Kino) (Sabo and Knezevic, 2019).

The *Eucalyptus* is among the most widely planted genera in the world, which successfully establish and spread globally as a result of its fast growth and easy adaptability (Coppen, 2002; Akin *et al.,* 2010). The Leaves of *the Eucalyptus camaldulensis* plant are grey-blue in colour, alternate, drooping, 8 to 22 cm in length, 1 to 2 cm in width, frequently sickle in shape, tapering, and short pointed at the base (Sabo and Knezevic, 2019).

The Eucalyptus leaves, other plant parts and essential oils are used as Aboriginal herbal medicine for many eras owing to their anti-inflammatory, antiseptic, and antipyretic properties as related by Jeane *et al.,* (2003). It is administered as a medication for respiratory and urinary tract infections caused by bacterial infectious agents and as medication for sore throat (Bruneton, 1999; Ayepola and Adeniyi, 2008). The essential oils from its leaves are applied in treating lung diseases, in addition to the use of *Eucalyptus* volatile oils as expectorants (Adeniyi *et al.,* 2006), while the topical ointments containing Eucalyptus oil have been applied topically for wound treatment and infection caused by fungal infections in Aboriginal medicines (Ayepola and Adeniyi, 2008). A decoction from the Eucalyptus plant has been administered for the treating diarrhoea/dysentery arising from enteric infections, mouth thrush, asthma, wound infections, asthma, eczema and athlete's foot and many more (Bala, 2006).

As related to the instances above, it is obvious from the literature that there exist numerous age-old traditional medicinal applications of the *Eucalyptus camaldulensis* plant for curing several infectious and non-contagious diseases. To find the justification for its usage in conventional medicine, This study intended to look at the antibacterial activity or potency of the *Eucalyptus camaldulensis* plant extract and screen for its essential chemical phyto-ingredients, as this will further expose its antimicrobial value which can be tapped by the pharmaceutical industries for the production of new line of potent drugs to combat antibiotic resistance.

MATERIALS AND METHODS

Collection of Plant Material, Preparation and Processing

The *Eucalyptus camaldulensis* plant materials such as the leaves and the stem barks were collected. The leaves and stem barks of the *Eucalyptus camaldulensis* plant were obtained at the plant garden of Gombe State University. These materials were taken to the herbarium in the Department of Plant Biology for taxonomical identification. The leaves and stem barks of the plant were initially processed by carefully cleaning the leaves and stem barks with sterile cotton wool to remove visible dirt, then stripping of leaves from their stems. Afterwards, the leaves were rinsed with distilled water before placing them on the drying frames. Air-drying method described by Ibrahim *et al.,* (2015) was adopted where the prepared *E. camaldulensis* plant materials were air-dried at room temperature for 12 to 14 days. The already dried leaves and the stem barks

of *E. camaldulensis* were further processed by grinding into a powdery form using the laboratory blender, subsequently, the powdery products of the leaves and stem barks were stored in a dry and airtight container before the extraction phase.

Ethanolic Extraction of *Eucalyptus camaldulensis*

The extraction using ethanol as solvent was achieved by adopting methods closely related to Parekh and Chanda (2007) and Fatope and Hamisu (1993). Fifty (50) grams of the pulverized *Eucalyptus camaldulensis* leaves and stem barks were separately macerated in 500 mL ethanol contained in a flask for 24 days (at room temperature) with consistent shaking. The mixtures were initially filtered with a muslin cloth, and further filtering was achieved with filter paper. The liquid collected was heated to evaporation to obtain an extract concentrate, and the crude leaf and stem bark extract obtained were preserved at 4 °C before being used for the antimicrobial susceptibility test.

Sterility Test on the Ethanolic Plant Extracts

The sterility test was achieved with the ethanolic leaf and stem bark extracts to verify the sterility of the extract intended for the susceptibility test. As described by Okolo *et al.,* (2019), the sterility test was carried out by adding 2 mL of the extract into 10 mL of broth (Muller-Hinton) and then placed in the incubator at 37 °C for 24 hours. The non-detection of turbidity or the detection of clearness of the Muller-Hinton broth after 24 hours means a sterile extract, while the presence of turbidity in the tubes due to microbial growth implies a non-sterile or contaminated extract.

Screening for Phytochemical Constituents

A series of qualitative phytochemical analyses were conducted on the stem bark and leaf extracts of *E. camaldulensis* to detect phytochemical substances that could qualify *E. camaldulensis* as antimicrobial. Using standard methodologies, the extracts of *E. camaldulensis* were screened for the availability of phytochemical compounds such as tannins and alkaloids as described by Ciulci, (1994); flavonoids and phenols as described by Alara *et al.,* (2017), while saponins and glycosides were analysed for by methods reported by Trease and Evans, (1989).

Collection of Clinical Isolates

The clinical isolates of *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella t*yphi and *Candida albicans* used in this study were collected from the Microbiology laboratory of the Federal Teaching Hospital, Gombe State. These test bacterial isolates were initially sub-cultured on nutrient agar by streak-plating technique and cultured in the incubator at 37 °C for 1 day to obtain discrete colonies. For *Candida albicans,* the purification procedure was carried out on Potato Dextrose Agar (PDA) with Chloramphenicol and cultured at 25 °C for 5 days for the pure culture.

Confirmation of the Test Clinical Isolates

For confirmation of the test clinical isolates collected for this study, standard microbiological methodologies were adopted as described below:

Gram Staining technique and Microscopy

Colonies of microbial test organisms from the purified culture plates were initially stained by the Gram staining protocol described by Cheesbrough (2005) then stained smears were viewed under the microscope at x100 objective to observe the microscopic morphology of the various test Isolates.

Confirmatory Biochemical Analyses

Additional confirmation of the test organisms (*Pseudomonas aeruginosa, Staphylococcus aureus,* and *Salmonella typhi*) was achieved via the various biochemical tests which include catalase,

coagulase, motility, urease, citrate utilization and indole test. Standard procedures for biochemical analyses were adopted as reported by Cheesbrough, (2002).

Germ Tube Test for the Confirmation of Candida albicans

A pure isolate of *Candida albicans* picked from the pure culture plate was subjected to a Germ tube fermentation test for genuine confirmation of the fungal isolate. The Germ tube fermentation test was achieved using the method described by Cheesbrough, (2006). About 0.5 mL of human serum was added to a test tube and then inoculated with the pure culture of *C. albicans* from the purified culture. Afterwards, the inoculated tube was allowed in the incubator at 37 °C for about 3 hours. After incubation, a drop of the serum-*C. albicans* mixture was transferred from the test tube onto a glass slide and then covered using a cover slip and then examined under the microscope for sprouting yeast cells (tube-like outgrowths from the cells) at x10 and x40 objective lens.

Inoculum Standardization

A loopful of the culture obtained from the purified culture of the test organism was inoculated in peptone water in a sterile test tube and then incubated overnight at the temperature of 37 °C. 1 mL from the overnight broth culture was transferred into another sterile test tube, then subjected to dilution with distilled water until it matches the turbidity of 0.5 McFarland standard (Cheesbrough, 2005).

Stock and Standard Concentrations of *Eucalyptus camaldulensis* Plant extracts

The method of Ibrahim *et al.,* (2015) was applied in preparing 100 mg/mL stock concentration of both extracts of *Eucalyptus camaldulensis,* by dissolving 100 mg of the extract in 1 mL of Dimethyl sulfoxide (DMSO). Other lower standards such as 80, 60 40 and 20 mg/mL concentrations were prepared in sterile bijou bottles via appropriate dilution with DMSO from the stock solution. Afterwards, the prepared concentrations were preserved at 4 °C for an antimicrobial susceptibility test.

Antimicrobial Susceptibility Test

The agar well diffusion technique was adopted for the *in vitro* sensitivity test to determine the antimicrobial activity of the stem bark and leaf extracts of *Eucalyptus camaldulensis* on the various test organisms. This susceptibility test was carried out as reported by the National Committee for Clinical Laboratory Standards (NCCLS) (1993). Approximately, 25 mL of prepared Mueller-Hinton agar was poured into empty Petri dishes under aseptic conditions and left to set. A swab stick was inserted inside the standardized inocula of the test isolates and then inoculated by swabbing the complete surface of the Mueller-Hinton agar to produce a uniform swabbing coverage. Six (6) wells were bored on the inoculated Mueller-Hinton agar with a sterile 6 mm borer. Then 0.2 mL of the various concentrations (100, 80, 60, 40 and 20 mg/mL) of the leaf and stem bark extracts were poured into separate wells while DMSO used as a diluent was deposited into the sixth well as blank control. Also, the Gentamycin antibiotic disc (10µg) was used as a positive control. All the plates were allowed in the laminar flow for several minutes for adequate absorption and diffusion of the extracts into the agar wells. Afterwards, the plates were incubated for 24 hours at 37 °C. After incubation, the zone of inhibition or clearance produced around the wells were observed and measured with a calibrated meter rule in millimetre. On the contrary, for the anti-fungal susceptibility test, Chloramphenicol-containing agar was used to prevent any bacterial contamination. In the same vein, 0.2 mL of the same stock and standard concentrations of the *E. camaldulensis* leaf extract was added into the six (6) equidistant wells on the inoculated Chloramphenicol-containing agar. However, no antifungal activity was tested with the stem bark extract on *C. albicans.*

Plates were allowed for proper diffusion of then incubated at 25 °C for 72 hours before determining the diameter zone of inhibition in millimetres. Antimicrobial susceptibility assay was done in triplicates.

Minimum Inhibitory Concentration (MIC) Bioassay

The broth dilution technique was adopted for MIC detection as described by Gutpe, (2006). Before the MIC assay, the stock concentration of 100 mg/mL of the extracts was subjected to double or 2-fold dilutions until five lower concentrations of 50, 25, 12.5, 6.25 and 3.13 mg/mL were obtained in separate bijou bottles. For the MIC determination, 1 mL of the new standard concentrations (50, 25, 12.5, 6.25 and 3.12 mg/mL) of the leaf and the stem bark extracts were pipetted into five different sterile test tubes, afterwards 1 mL of Muller-Hinton broth was added to all the five tubes to yield a final concentration of approximately 25, 12.5, 6.25, 3.13 and 1.56 mg/mL which were used for the MIC determination bioassay. Afterwards, 50 µL of the standardized inocula of each of the test isolates were introduced into the test tube and then gently vortexed before incubating at 37 °C for 24 hours. Also, five control tubes were set up containing only the leaf or stem bark extract and the Mueller-Hinton broth as previously described without inoculation with the test organisms then incubated along with others. After the 24-hour incubation, all the tubes were inspected for microbial growth by checking for turbidity and comparing them with the respective control tubes which automatically had no growth. Consequently, the tubes with no growth signified the organism was inhibited by the leaf or stem bark extract at that concentration. Hence, the lowest concentration of the extract of *E. camaldulensis* that inhibited the test microorganisms (i.e. no turbidity in the tubes) was recorded as the MIC.

Minimum Bactericidal Concentration (MBC)

A Minimum Bactericidal Concentration (MBC) was carried out to determine the least concentration of extracts that could kill the test microbes. The method described by Nwankwo and Chika, (2017) was applied for the MBC determination. The tubes with no sign of bacterial growth during the MIC determination were sub-cultured on Mueller-Hinton agar. The inoculated bacterial plates were then incubated at 37 °C for 24 hours, after which the MBC was determined as the least concentration of the leaf or stem bark extract that showed no visible colony growth on the Mueller-Hinton agar plate.

RESULTS

This study presents the results of the phyto-constituent screening of the leaf and stem bark extracts of *E. camaldulensis* and its antimicrobial activity against the test microorganisms namely, *S. aureus, S. typhi* and *P. aeruginosa.* Further antimicrobial assessment of *E. camaldulensis* extracts produced other results such as the MICs and the MBCs.

Table 1 is the qualitative result of the phytochemical screening of the leaf and stem bark extracts of *E. camaldulensis* which shows different phytochemical constituents such as the tannins, saponins, glycosides, alkaloids, flavonoids and phenols screened for in this study.

Table 1: Phytochemical Constituents of Leaf and Stem Bark Extract of *Eucalyptus camaldulensis*.

Phyto-constituents	Leaf extract	Stem bark extract
Tannins	+	+
Saponins	+	+
Glycosides	+	+
Alkaloids	+	+
Phenols	-	+
Flavonoids	-	+

Key: + means present, while – means absent

From Table 1, plant bioactive constituents such as tannins, saponins, glycosides and alkaloids were discovered in both the leaf and the stem bark extract of *E. camaldulensis,* while phenols and flavonoids were only present in the stem bark extract. Before this, the sterility test shows no microbial growth after the 24-hour incubation thus implies both extracts of *E. camaldulensis* used for this study were sterile.

Table 2 shows the susceptibility of the three test bacterial isolates to the varied concentrations of the leaf extract of *E. camaldulensis* via the production of zones of inhibition (ZI). Interestingly, the lowest zone of inhibition was 22 mm produced by the lowest concentrations of 20 mg/mL for all three test organisms. While the highest ZI were within the range of 38 to 40 mm produced at the highest concentration of 100 mg/mL with *S. aureus* and *P. aeruginosa* showing the most susceptibility to the leaf extract. The result in Table 2 also shows that these ZI produced by 100 mg/mL which is the highest concentration of the leaf extract of *E. camaldulensis* is closely contested with the ZI produced by the Gentamycin antibiotic.

Also, in Table 2, the lowest ZI recorded was within the range of 18 to 24 mm for the lowest concentration of stem bark extract of *E. camaldulensis.* As seen in the case of the leaf extract, the highest ZI observed for the stem bark extract of *E. camaldulensis* is 40 mm at the highest concentration (100 mg/mL) specifically for *P. aeruginosa.* Though *S. typhi* also produced the highest susceptibility here while *S. aureus* was next in highest. Similarly, the highest ZI produced by 100 mg/mL (highest concentration) of the stem bark extract of *E. camaldulensis* varies less with the Gentamycin antibiotic-positive control portraying a variation of ±2 mm.

Table 2: Antibacterial Activity of Leaf and Stem Bark Extract of *Eucalyptus camaldulensis* on Bacterial Isolates

Extract	Test Organisms	Zone of Inhibition (mm) for varied Conc. (mg/mL)						
		NC	20	40	60	80	100	GT
Leave	*S. aureus*	0	22	25	30	35	40	42
	S. typhi	0	22	24	29	30	38	40
	P. aeruginosa	0	22	26	35	37	40	43
Stem bark	*S. aureus*	0	24	29	29	33	38	40
	S. typhi	0	18	28	32	38	40	42
	P. aeruginosa	0	22	24	30	31	40	41

GT = Gentamycin antibiotic disc (10µg), NC = negative control/blank.

For the antifungal activity of only the leaf extract of *E. camaldulensis,* Table 3 shows 13 mm as the lowest ZI produced at the lowest concentration of 20 mg/mL of the leaf extract against *C. albicans* fungal isolate. While the highest concentration which was 100 mg/mL of the leaf extract of *E. camaldulensis* yielded only 27 mm as the ZI measured in mm. However, no antifungal drug was applied as the positive control here.

Table 3: Antifungal Activity of Leaf Extract of *Eucalyptus camaldulensis* on *Candida albicans*

Concentration (mg/mL)	0	20	40	60	80	100
Zone of Inhibition (mm)	0	13	21	24	25	27

Table 4 displays the qualitative result of the MIC bioassay where various lower standard concentrations of the *E. camaldulensis* extracts were applied for the determination of the concentrations that can inhibit microbial growth of the three test organisms, and from which the MIC was determined. Interestingly, for both leaf and stem bark extract, 6.25, 12.50 and 25.0 mg/mL standard concentration inhibited the growth of *S. aureus* (Table 4), as no turbidity was observed after 24 hours of incubation when checked with the controls. The same was observed with *P. aeruginosa* while only 12.5 and 25.0 mg/mL concentration of the leaf and stem bark was detected to inhibit the growth of *Salmonella typhi.*

Also, in Table 5, the various concentration of the leaf and stem bark extract of *E. camaldulensis* that caused bactericidal effects on the various test organisms are shown. The same concentrations (12.5 and 25.0 mg/mL) of the leaf and stem bark extract were bactericidal to *S. aureus* while only 25 mg/mL of the leaf and stem bark extract of *E. camaldulensis* caused a bactericidal effect on the *Salmonella typhi.* On the contrary, 12.5 and 25.0 mg/mL of

the leaf extract were bactericidal to *P. aeruginosa* while only the highest concentration of 25 mg/mL of the stem bark extract of *E. camaldulensis* produced a bactericidal effect on the *P. aeruginosa.*

Table 4: Qualitative Detection of Growth Inhibition of Bacterial Isolates by Extracts of *E. camaldulensis.*

Extract	Test organisms	Concentration (mg/mL)				
		25	12.5	6.25	3.13	1.56
Leave	*S. aureus*	-	-	-*	+	+
	S. typhi	-	-*	+	+	+
	P. aeruginosa	-	-	-*	+	+
Stem bark	*S. aureus*	-	-	-*	+	+
	S. typhi	-	-*	+	+	+
	P. aeruginosa	-	-	-*	+	+

Table 4 shows the qualitative result of the MIC bioassay where the negative sign (-) indicates the absence of turbidity in the tube signifying no growth of the test organisms, the positive sign (+) indicates the presence of turbidity in the tube due to the growth of the test organisms while the negative sign with an asterisk (-*) is the MIC which was the lowest concentration that inhibited the growth of the test organisms.

Table 5: Qualitative Detection of Bactericidal Concentrations of *E. camaldulensis* Extract against the Bacterial Isolates.

Extract	Test organisms	Concentration (mg/mL)		
		25.0	12.50	6.25
Leave	*S. aureus*	-	-*	+
	S. typhi	-*	+	+
	P. aeruginosa	-	-*	+
Stem bark	*S. aureus*	-	-*	+
	S. typhi	-*	+	+
	P. aeruginosa	-*	+	+

Table 5 shows the qualitative result of the MBC bioassay where the negative signs (-) indicate the absence of colonies on the Mueller-Hinton agar plate signifying no growth of the test organisms, the positive signs (+) indicate the presence of colonies on the Mueller-Hinton agar implying the growth of the test organisms while the negative sign with an asterisk (-*) was the MBC as the lowest concentration of the *E. camaldulensis* extract that kills the test organisms.

In determining the MIC, the lowest concentration which inhibits the growth (where no visual turbidity is observed) of microorganisms is regarded as the MIC (Lajubutu *et al.,* 1995). For this study, it is interesting to deduce from Table 6 that both the leaf and the stem bark extract of *E. camaldulensis* produced the same MIC against *S. aureus, S. typhi and P. aeruginosa.* Thus for both extracts, MIC against *S. aureus* and *P. aeruginosa* was 6.25 mg/mL

while the MIC that inhibited the growth of *S. typhi* was 12.5 mg/mL.

Also, both the leaf and the stem bark extract produced the same MBC against *S. aureus* and *S. typhi* (table 6) with MBCs of 12.5 and 25 mg/mL respectively. However, 12.5 mg/mL of the leaf extract of *E. camaldulensis* was the lowest bactericidal concentration against *P. aeruginosa* while a higher concentration (25 mg/mL) of the stem bark extract of *E. camaldulensis* was the MBC against the same bacterial isolate.

Table 6: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *E. camaldulensis* Extracts tested against the Bacterial Isolates.

Extract	Test organisms	MIC	MBC
Leaf	*S. aureus*	6.25	12.5
	S. typhi	12.5	25.0
	P. aeruginosa	6.25	12.5
Stem bark	*S. aureus*	6.25	12.5
	S. typhi	12.5	25.0
	P. aeruginosa	6.25	25.0

Table 6 shows the MIC and MBC of the leaf and stem bark extracts of *E. camaldulensis* against *S. aureus, S. typhi* and *P. aeruginosa.* MIC and MBC in mg/mL.

DISCUSSION

The leaf and stem bark extracts of *E. camaldulensis* portray antimicrobial activity when tested on the test microorganisms as established via the production of the zones of clearance. The zones of clearance exhibited consequently denote the susceptibility of the test organisms to different concentrations *of E. camaldulensis* plant extracts. Furthermore, the zone of inhibition observed, which depicted antimicrobial activity against the test organisms increases with an increase in the concentrations *of E. camaldulensis* extracts and vice versa; thus implying a concentration-dependent activity.

Findings from this study evidenced that both the leaf and the stem bark ethanolic extracts of *Eucalyptus camaldulensis* produced very high (comparison with the blank control) antimicrobial activity, especially against the bacterial isolates ranging from the lowest (20 mg/mL) to the highest concentration of the plant extracts. In addition, higher antibacterial activity was noticed compared to antifungal activity as 20 mg/mL which was the lowest concentration of the leaf extract yielded considerably higher zones of inhibition (22 mm) against bacterial isolates than exhibited with the fungal isolate which yielded significantly lower zone of clearance of 13 mm. However, the antifungal activity of the stem bark extract of *E. camaldulensis* on the *C. albicans* was not investigated due to the insufficiency of the stem bark extract. Also, there may not be a difference in the antibacterial potency of the stem bark and leaf extract of *Eucalyptus camaldulensis* as the zones of inhibitions or antibacterial activity produced by both extracts were closely contested even though no significance was tested. The antimicrobial potency of the leaf and stem bark extract of *Eucalyptus camaldulensis* observed in this study is in agreement with the antimicrobial activity of *E. camaldulensis* reported by Ayepola and Adeniyi (2008), Yano *et al.*, (2013) and

Azzah and Ibtisam, (2019). Specifically, Azzah and Ibtisam, (2019) reported a broad inhibitory activity of *the E. camaldulensis* plant on both Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and –negative bacteria (*E. coli* and *P. aeruginosa*).

Wojdylo *et al.,* (2007) stated that plants of medicinal value mostly contain bioactive molecules that react with microorganisms thereby inhibiting microbial growth as a result protecting the body against infectious disease agents. Accordingly, the antimicrobial activity of the *E. camaldulensis* is accredited to the presence of tannins, saponins, flavonoids and many other secondary metabolite compounds (Oyeleke *et al.,* 2008; Azzah and Ibtisam, 2019). Besides, among the mechanism or mode of action of tannins, is the capability to bind proteins consequently inhibiting microbial cell protein synthesis (Stern *et al.,* 1996). In corroborating these claims, the presence of several bioactive compounds is chiefly connected to the antimicrobial activity of *E. camaldulensis* plant extracts (Adesokan *et al.,* 2007; Ogbolie *et al.,* 2007; Owolabi *et al.,* 2007; Oyeleke *et al.,* 2008). As per this assertion, it is noteworthy, to state that this study also detected some bioactive phytochemical compounds such as tannins, saponins, glycosides, alkaloids, phenols and flavonoids in the stem bark extract of *E. camaldulensis;* while phenols and flavonoids were not detected in the leaf extract of the Red gum plant though others were detected.

The detection of these phytochemical compounds from the extracts of the *E. camaldulensis* plant is per other studies namely, Babayi *et al.,* (2004) and Abubakar, (2010) that confirmed the presence of different chemical phytoconstituents in *E. camaldulensis* plant or plant extract. The *E. camaldulensis* plant contains phyto-constituents such as sterols, alkaloids, glycosides, flavonoids, tannins and phenols (Abubakar, 2010; Sabo and Knezevic, 2019).

Also, for the stem bark extract, phyto-screening with methanol solvent signified the presence of flavonoids, saponins, volatile oils and tannins, and no detection of alkaloids, anthraquinones, glycosides and hydrolysable tannins (Islam *et al.,* 2014). Also, Ayepola and Adeniyi, (2008) reported the presence of saponins, cardiac glycosides and tannin in *E. camaldulensis* leaf extracts as secondary metabolites. The leaves of *E. camaldulensis* tested positive for tannins, flavonoids and sterols, specifically containing 5 to 11% tannin (Sabo and Knezevic, 2019), while the bark contains 2.5 to 16% tannin (Watt and Breyer-Brandwijk, 1962). However, as established by Sabo and Knezevic, 2019, the presence of these phyto-ingredients could vary depending on the type of plant materials, plant origin/location and even extraction solvents; as other studies have also reported the non-detection of some phytochemicals in the same Red gum plant extract. For instance, Jouki and Khazaei, (2010), stated that the methanol leaf extracts of the *E. camaldulensis* plant sampled in Iran contained tannins, saponins, balsam (gum) and volatile oils while lacking other phyto-components like flavonoid, hydrolysable tannin, glycosides, anthraquinones and alkaloid. On the contrary, Singh and Thakur, (2016), found flavonoids, anthraquinones, terpenoids and saponins in methanolic leaf extract of *E. camaldulensis* sampled in India while there was negative detection of tannins, cardiac glycosides, and alkaloids. Chuku *et al.,* (2016) reported the presence of moderate to high quantities of phytochemical metabolites such as saponins, alkaloids, flavonoids, tannins, steroids, cardiac glycosides and carbohydrates but the absence of anthraquinones from leaf extract of *E. camaldulensis* from Nigeria; though with different extraction solvents such as methanol, ethanol and petroleum ether. Consequently, phytochemical findings in this study largely agree with other studies though except for a few metabolites not

screened. Therefore, several factors could vary the phytochemical constituents of *E. camaldulensis* extract, as Cadahia *et al.,* (1997) clarified that extract of *Eucalyptus camaldulensis* are mostly rich in tannins and this varies both qualitatively and quantitatively based on the sample origin, also accordingly protoanthocyanidin amount was influenced by the geographical origin.

The detection of these important chemical phytoconstituents in the leaf and stem bark of *E. camaldulensis* extracts is an additional pointer to the pharmacological value of the plant and reinforced the therapeutic claims of the local traditional users. In this regard, it is indispensable to attribute the antimicrobial activity of the leaf and the stem bark extract of *the Eucalyptus camaldulensis* plant observed in this study to their bioactive phytochemical constituents detected in the plant extract. This corroborates with the study of Chuku *et al*., (2016) that reported *Eucalyptus camaldulensis* extracts possessed all the phytochemicals compounds screened for and therefore responsible for its antimicrobial activity.

Also, this study compared the antibacterial activity of the stem bark and leaf extract of *E. camaldulensis* with Gentamycin. Interestingly, the zone of inhibition produced by Gentamycin as compared with the 100 mg/mL of the leaf and stem bark extract is only in a difference of ±3 mm, though the antibiotic has the higher activity. Reasonably, a higher concentration of the extracts above 100 mg/mL may produce a higher antibacterial activity than the Gentamycin antibiotic. Azzah and Ibtisam, (2019) also reported the antimicrobial activity of *E. camaldulensis* at a broad spectrum level against both Gram-positive and -negative bacteria. This further relay a high level or broad antibacterial activity of *Eucalyptus camaldulensis* that could equal or even better some antibiotic drugs consequently calling for alternative antimicrobial agent; since drugs manufactured from plant and plant materials are safer than synthetic drugs of chemical origin (Abd El-Tawab *et al.,* 2017).

From the MIC result, though there was no difference in MIC produced for both leaf and stem bark extract of *E. camaldulensis,* the lowest MIC produced was 6.25 mg/mL against *S. aureus* and *P. aeruginosa*; and this again implies that lower concentration of the extracts still inhibited the growth of bacterial infectious agents as a result emphasizing its high antibacterial efficacy. Sabo and Knezevic (2019) reported higher MICs against Gram-negative bacteria in the range of 10 to 200 mg/mL, while the MIC against *P. aeruginosa* was in the range of 10 to 100 mg/mL. In corroboration to the findings in this study, MIC recorded against *S. typhi* is within the range reported by Sabo and Knezevic (2019). Nonetheless, Sabo and Knezevic (2019) later clarified the varied inhibitory concentrations from 0.08 µg/mL to 200 mg/mL stating that MIC depends on test organisms, antimicrobial properties of the plant and extraction method. For the MBC, concentrations higher than the MICs up to 25 mg/mL were detected to be bactericidal against the various infectious bacterial agents. Though, a lower MBC of 12.5 mg/mL was also detected as the lowest concentration of the *E. camaldulensis* extract that killed *S. aureus* and *P. aeruginosa.* By implication, this again expatiates on the high antibacterial activity of the *E. camaldulensis* plant extracts assessed. It must be acknowledged that MIC and the Minimum Fungicidal Concentration (MFC) of the leaf and stem bark extract of *E. camaldulensis* on the *C. albican* (fungal isolate) was not investigated in this study, nonetheless, this is highly recommended in future study.

CONCLUSION

The finding of this study revealed *E. camaldulensis* as having a wide range of antimicrobial activity against Gram-positive and –negative bacteria which is due to the

presence of rich phytochemicals. These validate its widely reported ancient and current usage in traditional and herbal medicines for the treatment of several illnesses. Future investigation on the exact mechanisms of actions of several phytochemicals will be beneficial for the development of new antimicrobial drugs.

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