GSC Biological and Pharmaceutical Sciences

eISSN: 2581-3250 CODEN (USA): GBPSC2

Cross Ref DOI: 10.30574/gscbps

Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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The phytochemical constituents and antibacterial activity of methanolic and ethanolic leaf and stem extracts of *Eucalyptus torelliana*, Nigeria

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GSC Biological and Pharmaceutical Sciences, 2021, 16(02), 306–311

Publication history: Received on 16 July 2021; revised on 22 August 2021; accepted on 25 August 2021

Article DOI: https://doi.org/10.30574/gscbps.2021.16.2.0246

Abstract

The use of traditional medicines has been observed to increase globally. The search for new antimicrobial agents has increased as a result of increase in microbial infections as well as antimicrobial resistance. The phytochemical and antibacterial activities of ethanolic and methanolic extract of leaves and stem of *Eucalyptus torelliana* was investigated to detect the presence of secondary metabolites and also evaluate their antibacterial potential.

The phytochemical constituents of the powdered leaves and stem of *Eucalyptus torelliana* were determined using standard methods. The antibacterial susceptibility of bacteria from different sources to the leaves and stem extract was determined using agar diffusion method. Minimum inhibitory concentrations, MIC of the extracts were also determined.

Phytochemical screening of *Eucalyptus torelliana* yielded glycosides, reducing sugars, condensed tannins and terpenoids in both leaf and stem extract while saponins were found only in the leaves extract. The ethanolic and methanolic extracts of *Eucalyptus torelliana* had antibacterial activities at 20mg/ml and 10mg/ml especially at 20mg/ml where it showed significant difference in their activity in relation to the negative control. The extracts from the stem were observed to have better antibacterial activity compared to the leaves. Gentamicin was used as a positive control. The ethanolic extracts had MIC range between 10mg/ml->10mg/ml while that of the methanolic extracts was >10mg/ml.

The results from this study validate the possible use of *Eucalyptus torelliana* in the production of new antimicrobial agents.

Keywords: *Eucalyptus torelliana*; Phytochemical screening; Antimicrobial activity; Minimum inhibitory concentration

1. Introduction

The genus *Eucalyptus* is a wide genus containing evergreen trees, shrubs and belongs to the order myrtales and family myrtaceae [1]. Hundreds of species have since been described and about 900 are currently recognized [2]. The genus is native to Australia with only a few species introduced into Nigeria among which is *Eucalyptus torelliana* [2]. *Eucalyptus* plant parts have been reported to control several diseases derived from microbial infections [3]. Eucalyptus leaves have been found to be traditionally useful as a powerful antiseptic and is indicated in relieving coughs and colds, sore throats, other respiratory tract infections, wounds, ulcers and urinary tract infections especially caused by bacteria [4, 5, 6]. Aqueous extracts of *E. torelliana* leaves are also reported to be traditionally useful as analgesic, anti-inflammatory and also relieves cancer-related symptoms and intestinal challenges [7].

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In Nigeria, other plants of the genus *Eucalyptus* have been reported indicated in the treatment of gastrointestinal disorders [1]. A previous report showed the leaves also to be therapeutically useful in the treatment of diseases of the lung and also effective on tuberculosis [8]. As a result of the rise in antibiotic resistance and the increasing incidence of the use of medicinal plant in therapy, this study was done to evaluate the reported usefulness of *Eucalyptus torelliana* especially its antimicrobial properties.

2. Methods

2.1. Plant identification and authentication

Fresh samples of *Eucalyptus torelliana* (ET) were obtained and identified at Forest Research Institute of Nigeria (F.R.I.N) Ibadan, Oyo state. Voucher specimen of the plant with reference number FHI 112940 was kept at herbarium section of same institute for further reference. However, the plants were dried at room temperature until they fully dried after which they were pulverized to obtain coarse powder. However, samples were stored in separate sterile bottles for extraction.

2.2. Extraction

100 g and 150 g each of the leaf and stem of ET were macerated for 72 hours and stirred occasionally during the period of the extraction. Filtration and concentration on a water bath (70˚C) was subsequently carried out and resultant extracts were stored in clean, sterile, well labeled glass tubes and kept in a refrigerator (4˚C) till further used.

2.3. Phytochemical screening

Screening for the presence of phytochemical constituents such as saponins, flavonoids, glycosides and reducing sugars, alkaloids, phlobatannins, phenolic compounds, terpenoids were performed as described by standard methods with slight modifications [9, 10].

2.4. Microorganisms

Gram negative bacteria from different sources (wound infections, respiratory tract infections, urinary tract infection and gastrointestinal intestinal tract) were collected from Federal Medical Center (FMC) Asaba in Delta state and used for the antimicrobial studies. All the isolates were maintained on nutrient agar slants at 4˚C.

2.5. Preparation of Media, Extracts and Standards

All media were prepared according to the manufacturer's directions. For susceptibility testing, 0.4 g of the various extracts was weighed and placed in respective universal bottles. 1 mL of dimethylsulphoxide (DMSO) was added in the bottles to dissolve the extracts and 19 mL of sterile distilled water was added to make up the volume to 20 mg/mL. 0.5 mL of the 20 mg/mL extract solution was collected and 0.5 mL of sterile distilled water was added to make a concentration of 10 mg/mL. This was carried out for all the extracts.

However, for minimum inhibitory concentration, 0.4 g of the extract was weighed and 1 mL of DMSO was added to dissolve the extracts. 19 mL of sterile distilled water was added to make up the volume to 20 mg/mL. A serial dilution was carried out to make 10, 5, 2.5, 1.25 and 0.625 mg/mL. This was carried out for all the extracts respectively. Preparation of the negative control was carried out by introducing 1 mL of DMSO into a universal bottle and 19 mL of sterile distilled water was added to the universal bottle to make a concentration of 5% DMSO. The positive control was prepared by introducing 1 mL of gentamicin (80 mg/2mL) into 99 mL of sterile distilled water to make 400 µg/mL. Then 1 mL for the 400 µg/mL was collected and introduced into 79 mL of sterile distilled water to make a final concentration of 5 µg/mL.

2.6. Determination of Antibacterial Activity

The agar diffusion technique was employed with slight modifications [11]. A 10^{-2} dilution of the collected isolates was prepared for all isolates. 0.1 mL of each isolate was introduced into the pre-prepared Muller Hinton Agar plates and surface plated using a sterile swab sticks. The sterile cork borer {8 mm diameter} was used to bore equidistant holes on the plates. Using a Pasteur pipette, 0.1 mL of the 10 and 20 mg/mL extracts were used to fill up the holes. 0.1 mL of the positive control and negative control were also used. The experiment was done in triplicates. The zone of inhibition was obtained using a calibrated ruler and noted, indicating positive results.

2.7. Evaluation of Minimum Inhibitory Concentration (MIC)

A previous method was used in evaluating the MIC [12]. Various concentrations of the extracts were prepared. 2 mL of the 20 mg/mL was added to MacCartney bottle containing 18 mL of already sterilized Muller Hinton Agar, and then the agar was poured to set. This was carried out for the other different concentrations of the four extracts. An overnight broth culture of the bacterial isolates was introduced on each plate using a wire loop. The plates were incubated for 24 hours and the minimum inhibitory concentration of the extracts noted.

3. Results

3.1. Phytochemical Analysis

Table 1 summarizes the qualitative phytochemical analyses of *E. torelliana*. Results showed the extracts under investigation to contain glycoside and reducing sugar, condensed tannins, terpenoids in both the leaves and stem while saponins were found only in the leaves.

Table 1 Phytochemical constituent of *E. torelliana*

	Leaf	**Stem**
Flavonoids	-	-
Saponin	+	-
Glycosides and reducing sugars	+	+
Alkaloids	-	-
Phloba-tannins	-	-
Condensed tannins	+	+
Terpenoids	+	+

Key: +- Present, - -Absent

3.2. Antibacterial Susceptibility

Susceptibility of the clinical isolates from various sources to the extracts of *E .torelliana* is presented in Table 2 and Table 3. The extracts and gentamicin used as the positive control were found to have significant activity on the isolates especially at 20 mg/mL compared to the negative control (5% DMSO).

Table 2 Antibacterial activities of the ethanolic extracts of *E. torelliana*

Organism	**Negative control 5% DMSO**	**Positive control 5 µg/mL Gentamicin**	**Leaf extract 10 mg/mL**	**Leaf extract 20 mg/Ml**	**Stem extract 10 mg/mL**	**Stem extract 20 mg/mL**
E. coli 1	0.00±0.00	11.00±0.00*	8.33±0.88*	12.33±0.33*	11±0.00*	12.67±0.33*
E. coli 2	0.00±0.00	10.00±0.00*	10.33±0.33*	13.33±0.33*	9.33±0.33*	12.33±0.33*
P. aeruginosa 1	0.00±0.00	10.00±0.00*	10.00±0.58*	13.33±0.33*	10.00±0.00*	12.33±0.33*
P. aeruginosa 2	0.00±0.00	10.00±0.00*	9.67±0.33*	11.33±0.33*	10.00±0.58*	13.00±0.00*
Klebsiella sp 1	0.00±0.00	17.00±0.00*	9.67±0.33*	13.67±0.88*	9.00±0.58*	11.33±0.33*
Klebsiella sp 2	0.00±0.00	13.00±0.00*	9.33±0.67*	12.67±0.67*	11.00±0.00*	13.00±0.00*
P. mirabilis 1	0.00±0.00	10.00±0.00*	11.00±0.00*	13.00±0.00*	10.67±0.33*	14.00±0.58*
P. mirabilis 2	0.00±0.00	12.00±0.00*	0.00±0.00	14.33±.0.33*	10.67±0.33*	14.00±0.00*
S. typhi 1	0.00±0.00	10.00±0.00*	9.67±0.33*	13.67±0.33*	9.00±0.00*	13.00±0.00*
S. typhi 2	0.00±0.00	11.00±0.00*	9.00±0.00*	13.00±0.00*	10.33±0.33*	13.67±0.33*

The values above are mean of three replicates n=3. Mean ± SEM. Values with superscript * indicates significant difference at P< 0.05 while values with no superscript * indicate no significant difference in relation to the negative control (5%DMSO)

3.3. MIC of the Stem and Leaf Extracts

Table 4 show the MIC of the extracts on the clinical isolates. The MIC of the ethanol extracts ranged between 10 > 10 mg/ml while that of the methanol extracts was >10mg/ml.

Table 3 Antibacterial activities of the methanolic extracts of *E. torelliana*

Organism	Negative control 5% DMSO	Positive control 5 µg/mL Gentamicin	Leaf extract 10 mg/mL	Leaf extract 20 mg/mL	Stem extract 10 mg/mL	Stem extract 20 mg/mL
E. coli 1	0.00±0.00	11.00±0.00*	12.00±1.53*	12.33±0.88*	10.67±0.33*	12.33±0.33*
E. coli 2	0.00±0.00	11.00±0.00*	9.67±0.67*	13.33±0.67*	10.67±0.33*	12.00±0.00*
P. aeruginosa 1	0.00±0.00	9.00±0.00*	10.33±0.33*	12.00±0.58*	8.33±0.00*	13.33±0.33*
P. aeruginosa 2	0.00±0.00	12.00±0.00*	0.00±0.00	13.00±0.00*	8.67±0.33*	14.67±0.33*
Klebsiella sp 1	0.00±0.00	16.00±0.00*	8.67±0.67*	14.33±0.33*	8.67±0.33*	13.67±0.33*
Klebsiella sp 2	0.00±0.00	9.00±0.00*	7.00±0.00*	11.00±0.00*	9.33±0.67*	12.67±0.33*
P. mirabilis 1	0.00±0.00	11.00±0.00*	0.00±0.00	15.00±0.58*	10.67±0.33*	12.33±0.33*
P. mirabilis 2	0.00±0.00	11.00±0.00*	10.67±0.33*	14.67±.0.33*	10.33±0.33*	12.67±0.00*
S. typhi 1	0.00±0.00	11.00±0.00*	11.33±0.33*	14.67±0.33*	10.33±0.33*	14.33±0.33*
S. typhi 2	0.00±0.00	10.00±0.00*	9.33±0.33*	13.00±0.58*	10.00±0.00*	12.67±0.33*

The values above are mean of three replicates n=3. Mean ± SEM. Values with superscript * indicates significant difference at P< 0.05 while values with no superscript * indicate no significant difference in relation to the negative control (5% DMSO)

Table 4 Minimum Inhibitory Concentration (MIC) determination of the ethanolic and methanolic extracts of *E. torelliana*

Organism	Ethanolic leaf extract (mg/mL)	Ethanolic stem extract (mg/mL)	Methanolic leaf extract (mg/mL)	Methanolic stem extract (mg/mL)
E. coli 1	>10.00	>10.00	>10.00	>10.00
E. coli 2	10.00	>10.00	>10.00	>10.00
P. aeruginosa 1	>10.00	>10.00	>10.00	>10.00
P. aeruginosa 2	10.00	>10.00	>10.00	>10.00
Klebsiella sp 1	10.00	>10.00	>10.00	>10.00
Klebsiella sp 2	10.00	>10.00	>10.00	>10.00
P. mirabilis 1	10.00	>10.00	>10.00	>10.00
P. mirabilis 2	>10.00	>10.00	>10.00	>10.00
S. typhi 1	10.00	>10.00	>10.00	>10.00
S. typhi 2	>10.00	>10.00	>10.00	>10.00

4. Discussion

Chemical and biological investigations of ethnomedicinal plants with high therapeutic indices and reputation of being curative have furnished the world with many clinically potent drugs [13, 14]. The phytochemical screening carried out on ET leaves showed the presence of saponins, condensed tannins, terpenoids, glycosides and reducing sugars while the stem of ET contained the same secondary metabolites except saponins. Alkaloids, flavonoids and phlobatannins were absent in both the leaves and the stem of ET. The phytochemical test results shows that the plant extracts contain

chemical constituents of pharmacological importance. The results of the phytochemical screening were in line with the results obtained by some other researchers like Adeniyi *et al*. [15] who reported the presence of saponins and tannins in the stem of ET and anthraquinnone, glycosides in addition to saponins and tannins were also reported in the leaves of ET. Adeniyi and Ayepola, [16] also reported the phytochemical constituents of ET leaf to include tannins, saponins, anthraquinone and cardiac glycosides. Tannins have been reported in the inhibition cell protein synthesis [17] Saponins detected in the leaf extract in this study have been reported to be important therapeutically as they are shown to have hypolipidemic and anticancer activity [18]. They are also important for the activity of cardiac glycosides [19]. Plants rich in saponins have also reported to have immune boosting and inflammatory properties [20]. The antibacterial activity detected in the study may be as a result of the above phytochemical constituents present. Previous reports reveal that phytochemicals show antimicrobial activity against a number of microorganisms [21, 22].

Results indicated that *E. torelliana* has good antimicrobial activity as it showed activity against the test isolates especially at 20mg/ml. The ethanolic and methanolic extracts of both the leaves and the stem at 10mg/ml and 20mg/ml showed a significant difference in their activity in relation to the negative control except at 10mg/ml of the ethanolic leaf extract on a *Proteus mirabilis* strain that showed no significant difference in relation to the negative control. Also, the methanolic leaf extract at 10mg/ml had no significant difference in its activity in relation to the negative control on a *Pseudomonas aeruginosa* and *Proteus mirabilis strain*. Results from the study indicated that an increase in the concentration of the plant extracts gives a reciprocal increase in the antibacterial activity of *Eucalyptus torelliana*. Stem extracts were observed to have better antibacterial activity compared to the leaves. The results obtained were in line with those obtained by Adeniyi *et al.* [1] who reported the crude extracts of *Eucalyptus torelliana* to have a good and broad spectrum of antibacterial activity. The results of the minimum inhibitory concentration confirm the antimicrobial screening test results. Minimum inhibitory concentrations of 10->10mg/ml were recorded for ethanolic and methanolic extracts of the leaf and stem of *E. torelliana*. This is in contrast to the results reported earlier by Adeniyi and Ayepola, [16] of lower MICs of *E. torelliana* extracts against test isolates. This contrast may be possibly due to the sources of the isolates used in this study. This shows that higher doses will be useful in infections indicated by these isolates.

5. Conclusion

This study confirms the historic use of *E. torelliana* and shows that methanolic and ethanolic extracts of *E. torelliana* can be useful as potential antibacterial agents. Further investigations that may represent possible sources of antimicrobials with biologically active compounds useful in the treatment of infections caused by bacteria especially the ones used in the study are recommended.

Compliance with ethical standards

Acknowledgments

We would want to acknowledge Mr Edafe Arusi for the technical support towards the success of this work.

Disclosure of conflict of interest

The authors (Christiana Jesumirhewe, Adaobi Sandra Okoro, Oluwasegun Adedokun) declare no conflict of interest/ competing interests.

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