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Antimicrobial Activity of *Emilia pratermissa* Leaf Extracts on Organisms Isolated from Patients with Otitis Media Attending Federal Medical Centre Owo and Ondo States Specialist Hospital Akure

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Authors' contributions

This work was carried out in collaboration between all authors. Author COA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AKO and POA managed the analyses of the study. Authors COA and AKO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The antimicrobial activity of methanol, hot water and cold water extracts of the leaf of *Emilia pratermissa* was evaluated against organisms isolated from patients with otitis media using agar well diffusion method. The isolated bacteria were subjected to antibiotic susceptibility testing using disc diffusion method. In addition, antifungal assay was conducted on the test fungi using agar well diffusion techniques. Minimum inhibitory concentration (MIC) was determined using the tube dilution technique. All the experiments were carried out in triplicates and data obtained from the study were subjected to analysis of variance (ANOVA). From the results, highest inhibition of methanol extract was recorded against *Staphylococcus aureus* with a zone of inhibition value of 29.00 mm while the least zone of inhibition value of 17.33 mm was recorded on *Streptococcus pyogenes.* For hot water

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extracts, the highest inhibition was recorded on *Streptococcus pneumoniae* with 24.67 mm and least inhibition on *Staphylococcus aureus* with 16.67 mm. For cold water extracts, the highest zone of inhibition was recorded for *Escherichia coli* with zone of inhibition value of 27.00 mm and lowest on *Proteus mirabilis* with 17.33 mm. For antifungal activity, methanol extract recorded the highest inhibition value of 11.67 for *Aspergillus niger* while the least zone of inhibition value of 7.33 mm was recored for *Candida albicans.*The highest inhibition by cold water extract was recorded for *Aspergillus flavus* with a zone of inhibition value of 12.33 mm while the least zone of inhibition value of 5.33 mm was recorded for *Candida albicans.* For hot water extract, *Aspergillus niger* had the highest zone of inhibition with 12.67 mm and least for *Candida albicans* with 3.33 mm. The minimum inhibitory concentrations (MICs) of extracts ranged from 3.125 mg/ml to 12.5 mg/ml for the test organisms. The antimicrobial activity of the three extracts inhibited the growth of the test organisms as much as that of the commercial antibiotics. Antimicrobial potential of *Emilia pratermissa* may be a source of new bioactive compounds for drug development, and also suggest that the plant could be promising in traditional phytomedicine for the treatment of otitis media infection.

Keywords: Antimicrobial activity; agar well diffusion; minimum inhibitory concentration; tube dilution.

1. INTRODUCTION

Plants have been described as gift of nature; they have been used as a therapeutic agent against various infectious diseases affecting both human and animals [1]. As such, much emphasis has been placed on the exploitation of medicinal plants that can be used in the treatment of infectious diseases [2]. The use of medicinal plants in folk medicine still serve as an alternative means of cost effective treatment of infections in covering the basic health needs of people in developing countries. The secondary metabolites (bioactive compounds) produced by these plants have been linked to their high medicinal potency and as such enable them to be used as a source of raw materials in the exploration of antimicrobial agents in the industry. Various plant parts, including herbs, spices, fruits, vegetables and tropical plants have been showed to contain these natural antimicrobials which are of intense medicinal benefits [3]. As more and more habitats of rich biodiversity are threatened by the forces of development, scientists all over the world are scrambling to identify new plant species and to learn about their traditional uses before they are lost forever.

Emilia pratermissa which belongs to the family of Asteraceae is a useful plant of west tropical Africa used generally as food and medicine for general healing*. Emilia praetermissa* milne-Redh (Asteraceae) was originally described from Sierra leone and Nigeria and was subsequently found in other West African countries, including Cameroun, Cote d' Ivoire, Ghana, Guinea and Liberia. It has similar uses as *Emilia lisowskiana* these are as follows; in West Africa and DR Congo, the leaves are occasionally eaten as a vegetable, either fresh in salads or cooked [3]. In Nigeria, the leaves are used to treat eye disorders, and also filariasis, the macerated leaves are used to treat heart problems and crushed leaves mixed with copper filings are used to dress ulcers. Also, a leaf decoction is used as a febrifuge, liquid in which the plant has been boiled is used to wash new-born babies. The green leaves are crushed and used externally to treat sores, sinusitis and as a poultice for wounds [4]. As a vegetable, the aim of this research is to determine the antimicrobial activity of *Emilia pratermissa* leaf extract on organisms isolated from patients with otitis media attending Federal Medical Centre Owo and Ondo States Specialist Hospital Akure.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of *Emilia pratermissa* Leaf Extract

Fresh leaves of *Emilia praetermissa* ("Odundun") used were harvested from the Federal University of Technology, Akure Campus and authenticated at the Herbarium of the Forestery Research Institute of Nigeria Ibadan. The leaves were rinsed with distilled water so as to remove dust and other foreign particules, and air dried for 4 weeks. The dried samples were then milled into powdery form using a clean mechanical blender and stored in sterile air tight plate container for further analysis. Three different solvents were used to extract the plant material with methanol (80%), hot water and cold water. Using methanol, hot and cold water for extraction, the

powdered materials (200 g and 100 g) were macerated separately for 72 hrs using methanol (80%), cold water and hot water (1000 ml). The mixtures were agitated after the addition of the solvent followed by sieving with a muslin cloth and filtered with No 1 Whatman filter paper. The filtrate was collected in a beaker and concentrated using rotary evaporator (Resona, Germany). All the extracts were kept at 4℃ in a refrigerator at least 24 hrs before subsequently testing.

Plate 1. Photograph showing *Emilia praetermissa* ("Odundun") in its natural habitat

2.2 Isolation of Microorganisms from the Clinical Samples

2.2.1 Isolation of bacteria

The plate streaking technique was used for isolation of microorganisms according to Cowan and Steel [5]. The swab samples were streak on the already solidified nutrient agar plate, blood agar and chocolate agar and incubated at 37℃. Pure cultures of isolates were obtained by sub-cultured onto freshly prepared plates as appropriate. Each colony of the isolate was picked and streaked on the plate in an aseptic condition using sterile inoculating loop and then incubated at 37℃ for 24 hours/48 h.

2.2.2 Isolation of fungal

The ear swabs were inoculated on plates containing sterilized Potato Dextrose Agar media by streaking method. The PDA plates were incubated at room temperature for 72 hours. After incubation the growth of fungi was observed and growth was subcultured to obtain pure culture. The morphology of the fungal isolates was observed. The colour and type of growth either woolly or cottony according to Devarshi et al. [6].

2.3 Test Isolates

The following bacterial and fungal were isolated from the samples and they are *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Streptococcus pneumoniae, Proteus mirabilis, Escherichia coli, Streptococcus pyogenes, Staphylococcus epidermidis,* and the fungal species were *Candida albicans, Aspergillus fumigatus, Aspergillus niger* and *Aspergillus flavus.*

2.4 Standardization of the Test Organisms

A loop full of test organism was inoculated on nutrient broth and incubated for 24 h. Exactly 0.2 ml from the 24 h culture of the organisms was dispensed into 20 ml sterile nutrient broth and incubated for 5 h to standardize the culture to 0.5 McFarland standards (10^6cfu/ml) before use according to the method of Oyeleke et al. [7].

2.5 Antibacterial Activity

2.5.1 Determination of antibacterial activities by leaf extract

The antibacterial activity of the plant extracts was determined using well diffusion technique as described by [19]. Hinton agar was then poured into different sterile petri dishes plates were swirled for even dispersion. Exactly one milliliter of each of the standardized test organism was transferred on the agar. The plates were swirled for even dispersion of the organisms. After solidification of the agar, a 6 mm diameter cork borer was used to make wells into each plate, about 50.0 mg/ml of each extracts were prepared with 20% Tween 20 used as reconstituting solvent was introduced. 20% Tween 20 was used as the negative control while Gentamycin served as the positive control, the plates were incubated at 37ºC for 24 h. Clear zones around the bored holes are indicative of the inhibition of the organisms by the extract. The activity indices (AI) were calculated as the division of zone of inhibition of the extract by that of the standard drug (Gentamycin).

2.5.2 Antibiotics sensitivity test

The disc diffusion method as described by Clinical Laboratory Standard Institute [8] was used to determine the effect of standard antibiotics on the bacterial isolates. Standard antibiotics disc were placed aseptically on agar plates already seeded with the test organisms using sterile forceps. The plates were then incubated at 37ºC for 24 hours. Zones of inhibition around the antibiotics disc were measured in millimeters.

2.6 Antifungal Screening of Plant Extracts of *Emilia praetermissa*

Antifungal activity by leaf extracts was determined by agar well diffusion technigue as described by [9]. About 50.0 mg/ml of each extracts were prepared with 20% Tween 20 used as reconstituting solvent. The experiment was conducted in triplicate. All the plates were incubated at 28℃ for 48 hours. Clear zones around the wells were measured in millimeters and ketoconazole was used as standard antifungal agent.

2.6.1 Antifungal sensitivity test

Antifungal sensitivity test was performed using agar well diffusion method as described by [9], antifungal activity of standard commercially produced antifungal agents against the test isolates was determined. PDA plates with approximately 20ml, cooled to 45℃ was poured into sterile Petri plates and left to solidify. An aliqout of culture (0.1 ml) was evenly spread on the surface of the solidified PDA plates. Wells of 4mm were bored in the agar with sterile Cork borers. Concentrations of 50 mg/ml of the fluconazole, ketoconazole, fuscin and clotrimazole were prepared. 0.2 ml of each antifungal drug was then introduced into each wells of appropriately labelled plates and holes with the aid of a micropipette.The plates were incubated at 26±1℃ for 24 to 72 hours. Inhibition zones were measured in triplicates.

2.7 Determination of Minimum Inhibition Concentration

Different concentrations of the extracts (50, 25, 12.5, 6.2, and 3.125 mg/ml) was prepared and assayed according to the method of [10]. The reconstituted extracts were serially diluted in sterile broth culture and 0.1 ml of the 18 hours broth culture of each of the test organisms that have been adjusted to turbidity equivalent to 0.5 McFarland standard was introduced to each test tubes containing the serially diluted extracts and incubated at 37℃ for 24 hours for bacteria and (28±2℃) for fungi respectively. After incubation, the tubes were examined for microbial growth by observing their turbidity, the MIC concentration was taken using spectrophotometer and the values were recorded.

2.8 Data Analysis

All the experiments were carried out in triplicate and data obtained from the study were subjected to analysis of variance. Treatment means were compared using Duncan's New Multiple Range Test (DNMRT) at 5% level of significance using SPSS version.

3. RESULTS AND DISCUSSION

The result of the antibacterial activities of the leaf extracts as shown in Table 1 reveals that the highest inhibition by the methanol extract was recorded with *Staphylococcus aureus* with a zone of inhibition value of 29.00 mm while the least zone of inhibition value of 17.33 mm was recorded on *Streptococcus pyogenes.* For hot water extracts, the highest inhibition was recorded on *Streptococcus. pneumoniae* with 24.67 mm and least inhibition on *Staphylococcus aureus* with 16.67 mm. For cold water extracts, the highest zone of inhibition was recorded for *E. coli* with zone of inhibition value of 27.00 mm and lowest on *Proteus mirabilis* with 17.33 mm. The extracts exerted good degree of inhibition against both the Gram negative and Gram positive organisms hence the extracts can be referred to as having a broad spectrum activity. For antifungal activity of the leaf extract in Table 2, methanol extract recorded the highest inhibition value of 11.67 for *A. niger* while the least zone of inhibition value of 7.33 mm was recored for *Candida albicans.* The highest inhibition by cold water extract was recorded for *A. flavus* with a zone of inhibition value of 12.33 mm while the least zone of inhibition value of 5.33 mm was recorded for *Candida albicans.* For hot water extract, *Aspergillus niger* had the highest zone of inhibition with 12.67 mm and least for *Candida albicans* with 3.33 mm.

The antimicrobial activity of *Emilia praetermissa* leaf extracts showed different zones of inhibition which were found to be organism and solvent

dependent. This may be as a result of observed difference in polarity of bioactive compounds that were extracted. Makanjuola et al. [11] also reported that antibacterial activities can be rationalized in terms of the polarity of the bioactive compounds to diffuse in the culture media used for visible susceptibility reaction by the test organisms to the extracts. The organic extract was found to possessed more potent antimicrobial activity than aqueous leaf extract of *Emilia praetermissa*. This may be because water is not efficient enough to extract inhibitory compounds from the plant extracts. The observed results showed that the organic extract (methanol) had higher antimicrobial activity compared to the aqueous extracts of *Emilia praetermissa* leaf. This is in line with the findings of Koduru et al. [12], Aliero et al. [13], Ashafa et al. [14], Aiyegoro et al. [15] and Ogundare et al. [16] that aqueous extracts of plants generally showed little or no antibacterial activities which were similar to our findings. Cowan [17] reported that the most active components are generally water insoluble, hence it is expected that low polarity organic solvents would yield more active extracts. Rios and Reccio [18] also said that the solvent used and the extraction system may both modify the final results of an experiment, which also explains the reason for the higher potency of the methanol extracts.

Table 3 reveals the antifungal sensitivity of some selected fungal isolates; all the organisms were inhibited by clotrimazole with the highest zone of inhibition of 14mm for *Aspergillus fumigatus,* while fluconazole had the highest inhibitory effect of 31.33 mm for *Aspergillus niger* and no effect on *Candida albicans*. All the organisms were inhibited by ketoconazole with the highest inhibitory effect of 26.33 mm for *Aspergillus fumigatus.* However, fuscin had no inhibitory effect on the fungal isolates. The commercial antibiotics were observed to be more effective in inhibiting the test organisms as shown on Table 3. Doughari et al. [19] reported that the state of administration of an antimicrobial agent affects the effectiveness of such agent, and that antibiotics being in a refined state and plant extracts in crude state, may record higher antimicrobial activity. Also, the small molecular size possessed by antibiotics as reported by Mailard [20] aids their solubility in diluents as this could enhance their penetration through the cell wall into the cytoplasm of the organism. However, the antimicrobial activities of the crude leaf extracts well with standard antibiotics, which if purified may exhibit higher zones of inhibition on the test organisms and may serve as a substitute to the commercially available antibiotics to which bacteria are developing resistant.

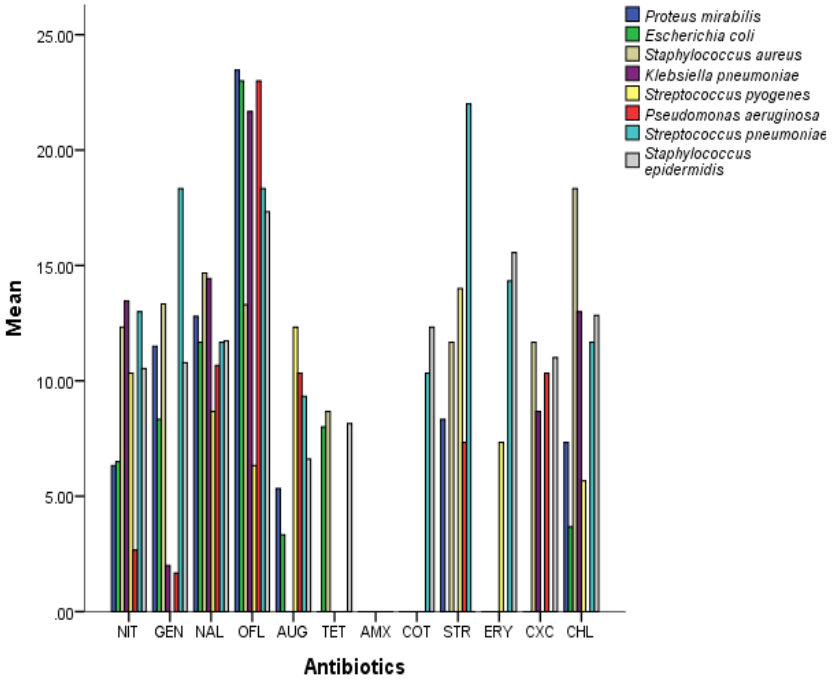

Fig. 1. Antibiotics sensitivity patterns of bacterial isolates

Table 1. Antibacterial activity (mm) of *Emilia praetermissa*

Extract	Selected isolates							
	Staphylococcus epidemidis	***Escherichia coli***	***Klebsiella pnuemoniae***	***Proteus mirabilis***	***Staphylococcus aureus***	***Streptococcus pneumoniae***	***Streptococcus pyogenes***	***Pseudomonas aeruginosa***
Methanol	21.67±0.88a	22.00±0.58b	19.00±0.58a	17.67±0.88a	29.00±0.58c	26.00±0.58a	17.33±0.88a	22.33±0.88b
Hot water	22.33±0.88a	18.67±0.33a	19.67±1.20a	20.67±0.33b	16.67±0.88a	24.67±0.88a	20.33±0.88b	22.00±0.58b
Cold water	22.00±1.15a	27.00±0.5.88c	21.67±0.88a	17.33±0.33a	20.67±0.67b	23.67±0.88a	22.00±0.58b	15.00±0.58a

Data are presented as Mean±S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05). Each value is a mean of 3 replicates

Table 2. Antifungal activity (mm) of *Emilia praetermissa*

Extract	Selected isolates			
	Candida albicans	***Aspergillus niger***	***Aspergillus flavus***	***Aspergillus fumigatus***
Methanol	7.33±0.33c	11.67±0.33b	14.67±0.33c	10.33±0.33b
Hot water	3.33±0.33a	12.67±0.33b	7.67±0.33a	9.33±0.33b
Cold water	5.33±0.33b	6.33±0.33a	12.33±0.33b	7.33±0.33a

Data are presented as Mean±S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05). Each value is a mean of 3 replicate

Table 3. Diameter of inhibition zones induced by selected antifungal agents

Antifungal agent	***Aspergillus niger***	***Aspergillus fumigates***	***Aspergillus flavus***	***Candida albicans***
Fluconazole	31.33 ± 0.88^{d}	29.67 ± 0.33^{d}	13.67 ± 0.33^{c}	0.00 ± 0.00^{a}
Ketoconazole	15.33 ± 0.57^{c}	26.33 ± 0.33^{c}	10.67 ± 0.33^{b}	9.00 ± 0.58^{b}
Fuscin	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Clotrimazole	11.67 ± 0.33^{b}	14.00 ± 0.58^{b}	10.67 ± 0.33^{b}	11.33 ± 0.33^{c}

Data are presented as Mean±S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05). Each value is a mean of 3 replicates

Table 4. Minimum inhibitory concentration (MIC) of bacterial isolates on leaf extract of *Emilia praetermissa*

Organisms	Methanol (mg/ml)	Cold water (mg/ml)	Hot water (mg/ml)
Saphylococcus aureus	12.5	6.25	3.125
Klebsiella pneumoniae	6.25	6.25	3.125
Pseudomonas aeruginosa	6.25	3.125	3.125
Escherichia coli	12.5	3.125	12.5
Staphylococcus epidermidis	3.125	3.125	3.125
Streptococcus pneumoniae	3.125	6.25	3.125
Streptococcus pyogenes	6.25	3.125	6.25
Proteus mirabilis	3.125	6.25	6.35

Table 5. Minimum inhibitory concentration (MIC) of fungal isolates on leaf extract of *Emilia praetermissa*

Extract (mg/ml)	*Candida albicans*	*Aspergillus flavus*	*Aspergillus fumigatus*	*Aspergillus niger*
Methanol	12.5	6.25	3.125	12.5
Cold water	6.25	3.125	3.125	6.25
Hot water	3.125	3.125	3.125	3.125

The MIC tests of the leaf extracts on the bacterial isolates as shown on Table 4 indicated that the three extracts (methanol, hot and cold water) were very active on the test organisms. The MIC of the extracts ranged from 3.125mg/ml to 12.5mg/ml. Methanol extract recorded the MIC value of 3.125 mg/ml for *Staphylooccus epidermidis, Streptococcus pneumoniae* and *Proteus mirabilis* while cold water extract had the MIC value of 3.125 mg/ml for *Pseudomonas aeruginosa, Escherichia coli* and *Staphylooccus epidermidis*. Hot water recorded the MIC value of 3.125 mg/ml for *Staphylooccus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylooccus epidermidis* and *Steptococcus pneumoniae respectively.* Table 5 illustrates the MIC values of *E. praetermissa* leaf extracts on fungal isolates, methanol extract had MIC value of 3.125 mg/ml for *Aspergillus fumigatus,* cold water extract recorded the minimum value of 3.125 mg/ml for *Aspergillus flavus* and *Aspergillus fumigates,* while hot water extract recorded the MIC value of 3.125 for *Aspergillus flavus*, *Aspergillus fumigatus, Aspergillus niger* and *Candida albicans.*

4. CONCLUSION

The leaf extract of *Emilia praetermissa* exhibited a good antimicrobial effect on the test isolates, the results of antimicrobial activity of *Emilia praetermissa* indicate the antimicrobial potential of the leaf extract which is a promising development that will help to discover new classes of antibiotics which might be used in the treatment of otitis media infections that otherwise have become resistant to the conventional antibiotics. Hence further purification of the extract and identification of the active component is necessary to enhance greater antimicrobial potency.

CONSENT

All consent form was duly signed by patients/guardian of the patients before samples collection.

ETHICAL APPROVAL

Ethical approval for the study was obtained from Ondo State Health Research Ethics Committee (OSHREC), Ministry of Health, Nigeria and Reasearch Ethic Committee of the Federal Medical Center Owo with reference numbers G.8061/104, FMC/OW/380/VOL.XXVIII/42.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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