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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.

ABSTRACT

The antimicrobial activities 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 result, 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 pyogens.* For hot water extracts, the highest inhibition was recorded on *Strep. pneumoniae* with 24.67mm and least inhibition on *S. aureus* with 16.67mm. For cold water extracts, the highest zone of inhibition was recorded for *E. coli* with zone of inhibition value of 27.00mm and lowest on *P. mirabilis* with 17.33mm For antifungal activity, , methanol extract recorded the highest inhibition value of 11.67 for *A. niger* while the least zone of inhibition value of 7.33mm was recored for *C. albicans.*The highest inhibition by cold water extract was recorded for *A. flavus* with a zone of inhibition value of 12.33mm while the least zone of inhibition value of 5.33mm was recorded for *C. albicans.* For hot water extract, *A. niger* had the highest zone of inhibition with 12.67mm and least for *C. albicans* with 3.33mm. The minimum inhibitory concentrations (MICs) of extracts ranged from 3.125mg/ml to 12.5mg/ml. 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, Agar well diffusion, Minimum inhibitory concentration, Tube dilution and Zone of inhibition

1.0 INTRODUTION

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Plants have been described as gift of nature; they have been used as a therapeutic agent against various infectious diseases affecting both human and animals1. As such, much emphasis has been placed on the exploitation of medicinal plants that can be used in the treatment of infectious diseases2. 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 (bio active 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 benefits3. 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 arelost 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 (Burkil, 2004).In Nigeria, the leaves are used to treat eye disorders, and also filariasis (Burkil, 2004), 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 (Burkil, 2004). The green

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leaves are crushed and used externally to treat sores, sinusitis and as a poultice for wounds (Ruffo *et al*., 2002). 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.

2.0 MATERIALS AND METHODS

2.1 Collection and Preparation of *Emilia pratermissa* leaf extract

Fresh leaves of *Solanum nigrum* ("Odu") (Black nightshade) 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 viz methanol (80%), hot water and cold water. Using methanol, hot and cold water for extraction, the powdered materials (200g, 100g) were macerated separately for 72 hrs using methanol (80%), cold water and hot water. 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 $4^{o}C$ in a refrigerator at least 24hrs before subsequently testing.

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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 Cowan and Steel [6]. The swab samples were streak on the already solidified nutrient agar plate, blood agar and chocolate agar and incubated at 37°C. 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; it was then incubated at 37°C for 24hours/48hours.

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2.2.2 Isolation of fungal

The ear swabs were inoculated on plates containing sterilised 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 sub cultured to obtain pure culture. The conial morphology of the fungal isolates was observed. The colour and type of growth either woolly or cottony was observed Devarshi *et al*. [8].

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, Strep.pyogens, Strep.pneumoniae, S. epidermidis,* and the fungal species were *Candida albicans, Aspergillus fumigatus, A. niger* and *A. 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 3- 5h to standardize the culture to 0.5 McFarland standards (106cfu/ml) before use according to the method of Oyeleke *et al*. [13].

2.4 Antibacterial activity

2.4.1 Determination of antibacterial activities by leaf extract

The antibacterial activity of the plant extracts was determined using well diffusion technique as described by [12]. Hinton agar was then poured into 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

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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 re- constituting 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 [1].

2.4.2 Antibiotics sensitivity test

The disc diffusion method as described by Clinical Laboratory Standard Institute [5] 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.

2.5 Antifungal screening of plant extracts of *Solanum nigrum*

Antifungal activity by leaf extracts was determined by agar well diffusion technigue as described by [15]. About 50.0 mg/ml of each extracts were prepared with 20% Tween 20 used as re- constituting solvent. The experiment was conducted in triplicate. All the plates were incubated at 28°C for 48 hours. Clear zones around the wells were measured in millimeters. ketoconazole was used as standard antifungal agent.

2.5.1 Antifungal sensitivity test

Antifungal sensitivity test was performed by agar well diffusion method as described by [15] was used to determine the antifungal activities of standard commercially produced antifungal agents against the test isolates. patato dextrose agar (PDA) approximately 20ml,

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cooled to 45°C was poured into sterile Petri plates and left to solidify. An aliqout of culture (0.1ml) 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 50ml/ml of the fluconazole, ketoconazole, fuscin and clotrimazole were prepared. 0.2ml 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°C for 24 to 72 hours. Inhibition zones were measured in triplicates.

2.6 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 [9]. The reconstituted extracts were serially diluted in sterile broth culture and 0.1ml of the 18hours broth culture of each of the test organisms that have been adjusted to turbidity equivalent to 0.5 McFarland standard was introduced to each teast tubes containing the serially diluted extracts and incubated at 37°C for 24 hours at room temperature. (28$\pm$2 ^{0}C) for bacteria and 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.

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.

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

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recorded on *Streptococcus pyogens.* For hot water extracts, the highest inhibition was recorded on *Strep. pneumoniae* with 24.67mm and least inhibition on *S. aureus* with 16.67mm. For cold water extracts, the highest zone of inhibition was recorded for *E. coli* with zone of inhibition value of 27.00mm and lowest on *P. mirabilis* with 17.33mm. The result shows that methanol extract possessed more potent antibacterial activity than the cold water extract and the hot water extract. 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 7.33mm was recored for *C. albicans.*The highest inhibition by cold water extract was recorded for *A. flavus* with a zone of inhibition value of 12.33mm while the least zone of inhibition value of 5.33mm was recorded for *C. albicans.* For hot water extract, *A. niger* had the highest zone of inhibition with 12.67mm and least for *C. albicans* with 3.33mm. 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 *A. fumigatus,* while fluconazole had the highest inhibitory effect of 31.33mm for *A. niger* and no effect on *C. albicans*. All the organisms were inhibited by ketoconazole with the highest inhibitory effect of 26.33mm for *A. 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*. [9] 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 [10] 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

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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.

Table 1: Antibacterial activity of *Emilia praetermissa*

Extract	Selected isolates							
	Staphylococcus epidemidis	*Escherichia coli*	*Klebsiella pnuemoniae*	*Proteus mirabilis*	*Staphylococcus aureus*	*Streptococcus pneumoniae*	*Strep.pyogens*	*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.

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Table 2: Antifungal activity 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 fumigatus*	*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

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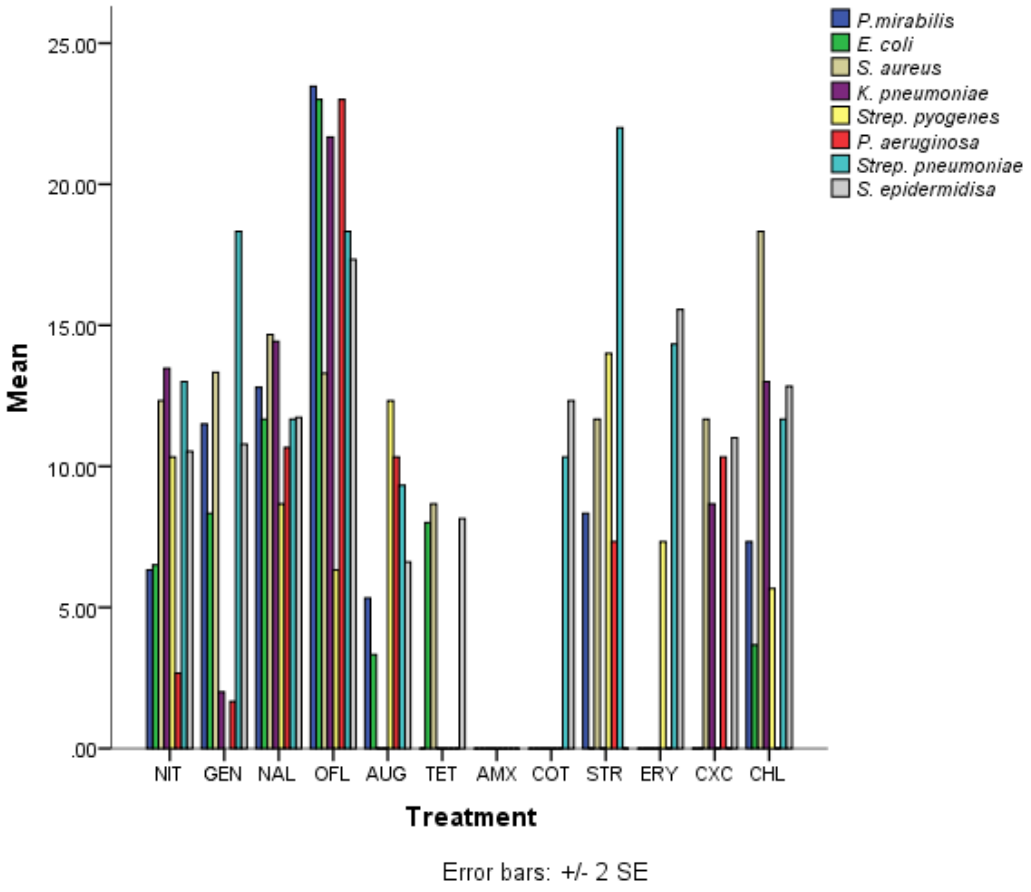

Fig1. Antibiotics sensitivity patterns of bacterial isolates

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. The minimum inhibitory concentration (MIC) tests of the leaf extracts on the bacterial isolates as shown on Fig 2 -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 3.125mg/ml for *S. epidermidis, Strep. pneumoniae* and *P. mirabilis* while cold water extract had the minimum inhibitory value of 3.125mg/ml for *P. aeruginosa, E. coli* and *S. epidermidis*. Hot water recorded the MIC value of 3.125mg/ml for *S. aureus, K. pneumoniae, Pseudomonas aeruginosa, S. epidermidis* and *Step. pneumoniae respectively.* Fig 5-7 illustrates the minimum inhibitory values of *E. praetermissa* leaf extracts on fungal isolates, methanol extract had MIC value of 3.125mg/ml for *A. fumigatus,* cold water extract recorded the minimum value of 3.125mg/ml for *A. flavus* and *A. fumigates,* while hot water extract recorded the MIC value of 3.125 for *A. flavus*, *A. fumigatus, A. niger* and *C. albicans.*

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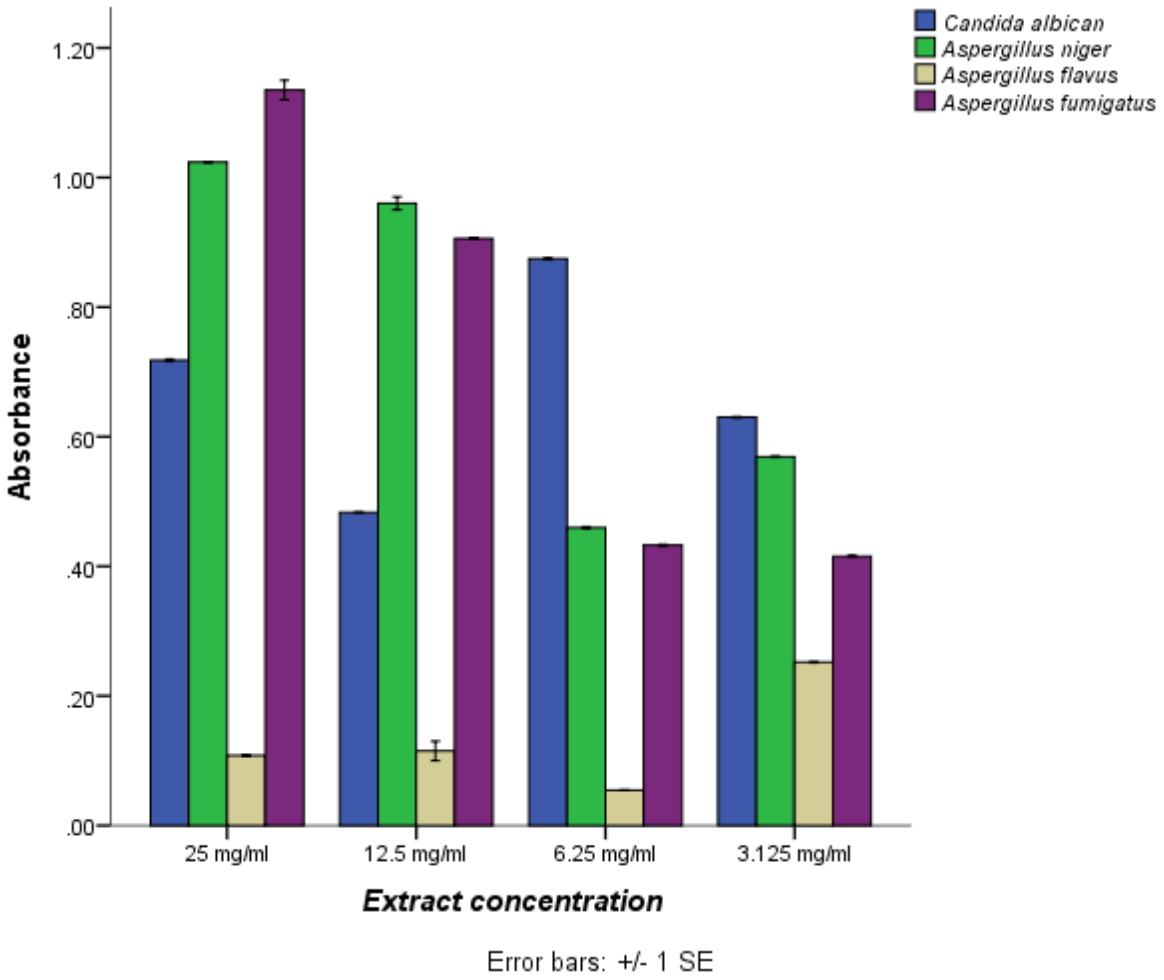

Fig2. Minimum Inhibitory Concentration of Methanolic extract of *Emilia praetermissa*

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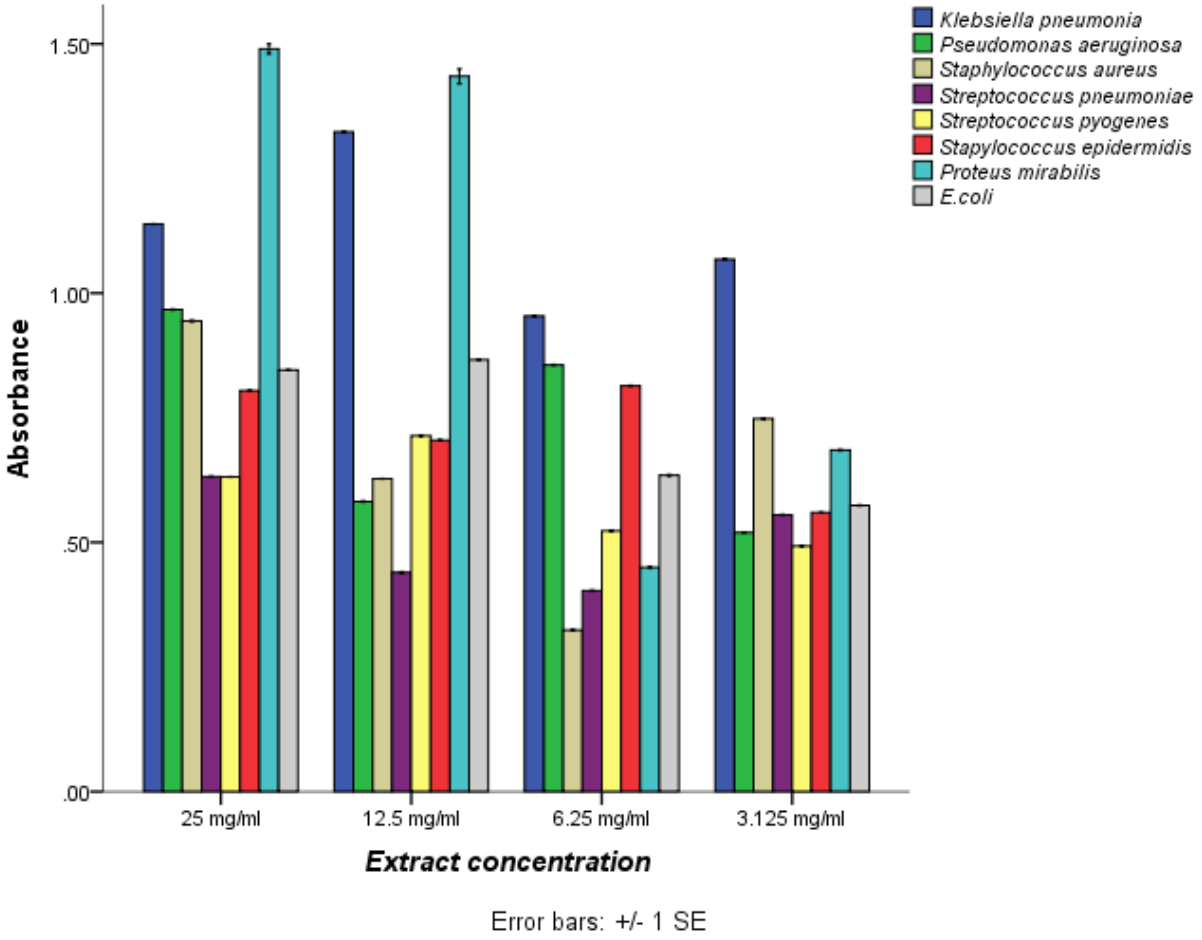

Fig3. Minimum Inhibitory Concentration of Cold water extract of *Emilia praetermissa*

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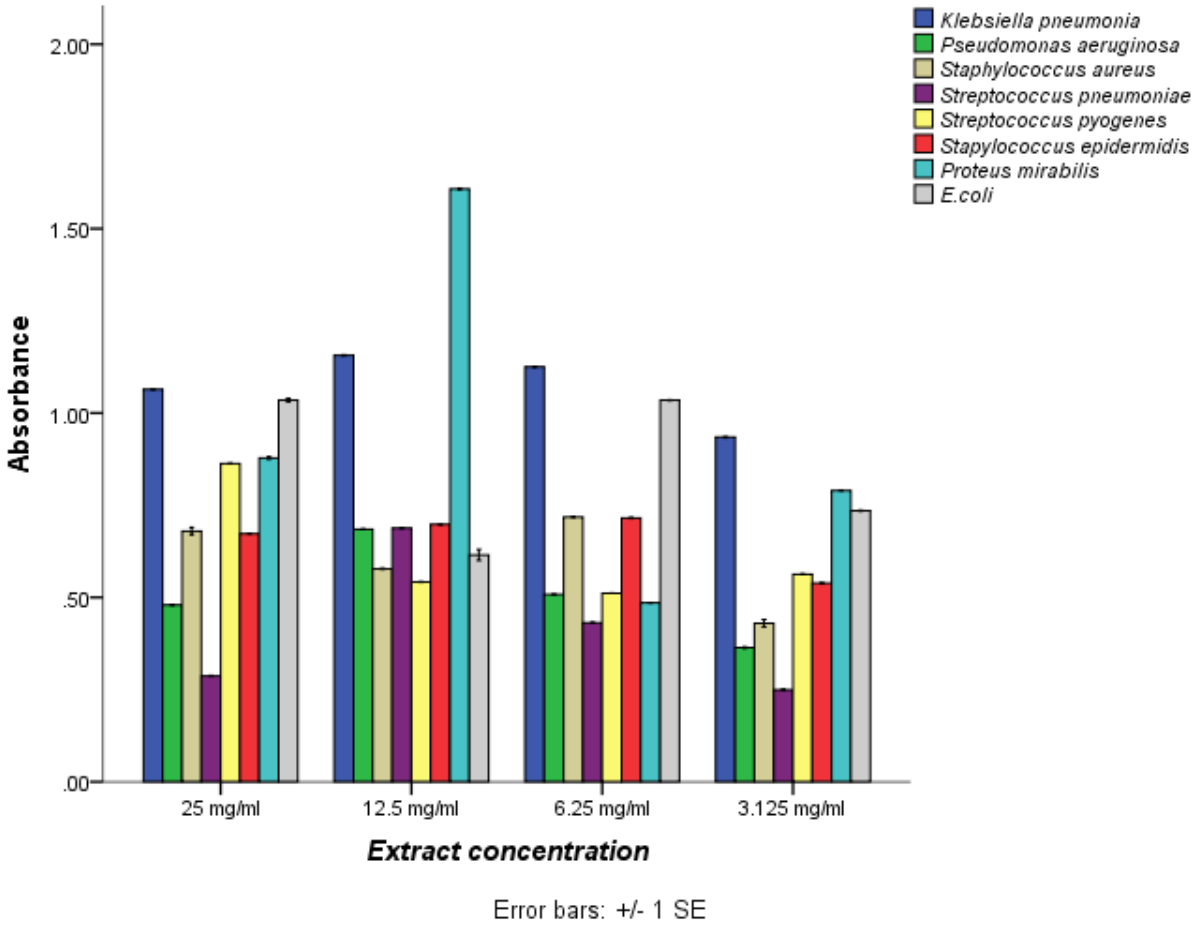

Fig4. Minimum Inhibitory Concentration of Hot water extract of *Emilia praetermissa*

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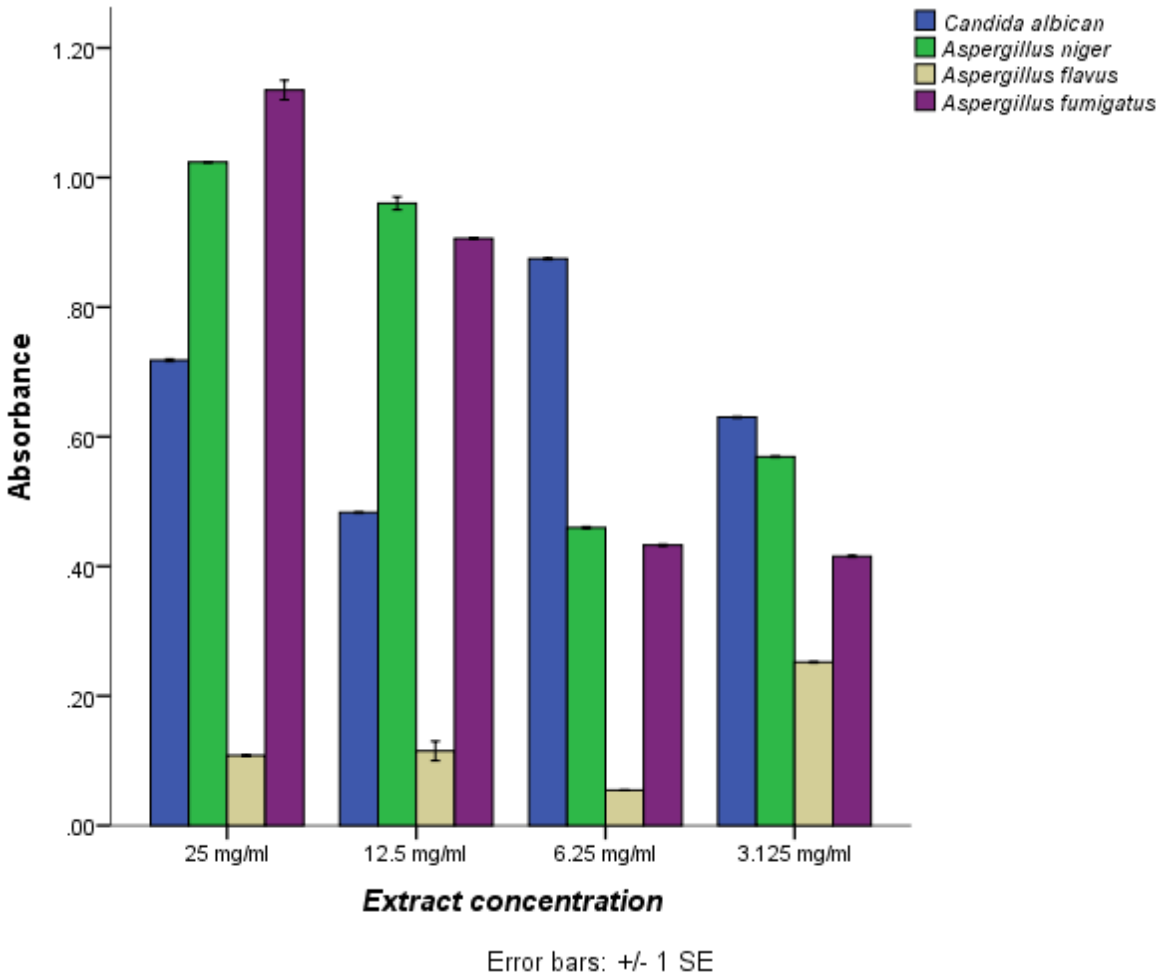

Fig5. Minimum Inhibitory Concentration of Methanolic extract of *Emilia praetermissa*

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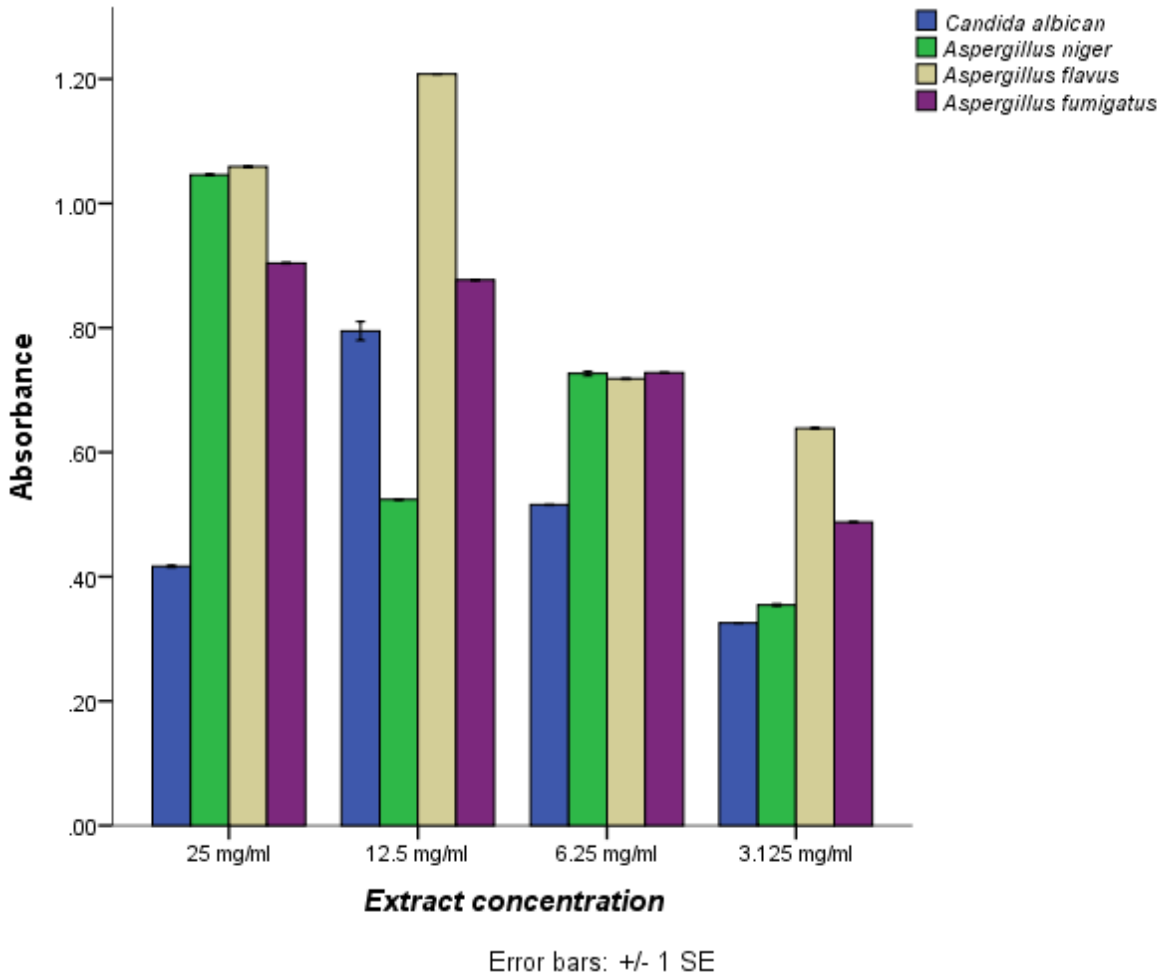

Fig6. Minimum Inhibitory Concentration of Hot water extract of *Emilia praetermissa*

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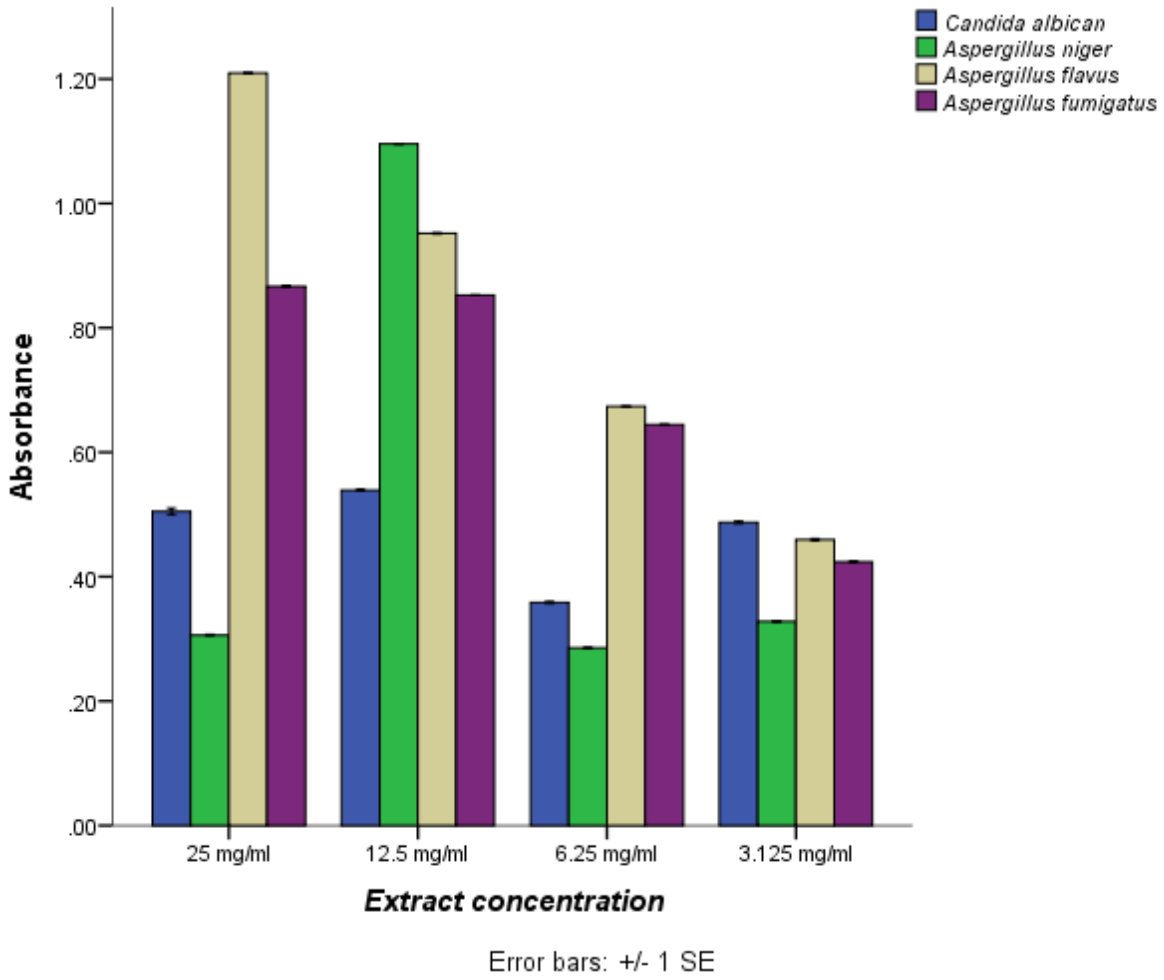

Fig7. Minimum Inhibitory Concentration of Cold water extract of *Emilia praetermissa*

CONCLUSION

The leaf extract of *Emilia praetermissa* exhibited a good antimicrobial effect on the test isolates. The availability and accessibility to plant makes the use of *Emilia praetermissa* a cost effective alternative medicine to the commercial antibiotics to which most organisms are now developing resistance. Further purification of the extract and identification of the active component is necessary to enhance greater antimicrobial potency. Herbal medicine has proven to be of great

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importance to the treatment of basic human diseases from time immemorial, this natural endowment (plants) should be exploited scientifically so as to tackle health related issues.

CONSENT

Applicable

ETHICAL APPROVAL

Applicable

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