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Invitro Antimicrobial Activity of *Erythropheleum suaveolens* Brenan and *Citrullus colocynthis* (L.) Schrad

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Abstract*—The increasing trend of bacterial and fungal diseases in susceptible people and drug resistance have resulted into an intense investigations into herbal medicines for their better efficacy and fewer side effects. Erythrophleum suaveolens Brenan (Family Fabaceae) and Citrullus colocynthis (L.) Schrad (Family Cucurbitaceae) are used traditionally as purgative, carminative, antirheumatic, anthelmintic, anticancerous and as a remedy for skin infection.This study investigated the antibacterial and antifungal activities of methanol extract of E. suaveolens and aqueuos extract of C. colocynthis against bacterial isolates (Escherichia coil, Pseudomonas aeruginosa, Staphylococcus aureus, and Bacillus cereus) and fungal isolates (Aspergillus niger, Aspergillus flavus, Trychophyton rubrum and Candida albican) using modified agar well diffusion technique at four four different concentrations. The antimicrobial screening of C. colocynthis revealed inhibitory activity against the fungal isolates with highest zone of inhibitory growth (15 mm) against A. terreus and A. niger (15 mm) and lowest inhibitory growth against T. rubrum (12 mm). C. colocynthis revealed highest inhibitory growth against B. cereus (15 mm) and lowest inhibitory growth against S. aureus (10 mm) while E. suaveolens revealed highest inhibitory activity against S. aureus (12 mm) and lowest against P. aeruginosa (8 mm) and B. cereus (8 mm).The extract showed highest inhibition against A. niger (12 mm) and lowest against C. albican (10 mm). E. suaveolens extract showed no inhibition against T. rubrum. The MIC and MBC of the C. colocynthis extract against the bacterial isolates ranges from 25 to 50 mg/ml and 50 to 150 mg/ml respectively while MIC and MFC ranges from 50 to 100 mm and 200 mg/ml respectively.The MIC and MBC of E. suaveolens against the bacterial isolates were from 50 to 100 mg/ml and 100 to 200 mg/ml respectively while MIC and MFC revealed 50 to 100 mg/ml and 200 mg/ml against the A. flavus, and C.albican. C. colocynthis and E. suaveolens extracts demonstrated inhibitory and lethal effects on common pathogenic bacteria and fungi but C. colocynthis extract had higher activity against selected microorganisms than E. suaveolens extract. This study validates the use of these plants in traditional medicine.*

***Keywords*—** *Citrullus colocynthis, Erythrophleum suaveolens, Antibacterial activity, Antifungal activity, Clinical isolates.*

I. Introduction

The global increase in antimicrobial resistance has intensified the need for new antimicrobial agents, particularly from natural sources such as medicinal plants, which are rich in bioactive secondary metabolites with diverse pharmacological properties. *In vitro* antimicrobial assays provide a controlled and reproducible platform to assess the inhibitory effects of plant extracts against clinically relevant pathogens and offer critical preliminary evidence supporting the therapeutic potential of ethnomedicinal species (Balouiri *et al*., 2016). *E. suaveolens* (Fabaceae), commonly known as the "ordeal tree," is a tropical African species traditionally used in folk medicine to treat infections, skin diseases, dysentery, and as an external disinfectant (Sonibare *et al*., 2023). *C. colocynthis* (L.) Schrad (Cucurbitaceae), commonly referred to as bitter apple, is another medicinal plant extensively used in traditional healthcare systems for infectious and inflammatory disorders (Shafaei *et al*., 2012). This study assessed the in vitro antimicrobial activity of *E. suaveolens* and *C. colocynthis* extracts against *E. coil, P. aeruginosa, S. aureus, B. cereus, A. niger, A. flavus, T. rubrum* and *C. albican* using agar-well diffusion method.

II. Materials and Methods

Preparation of Plant Samples

The whole fruits of *C. colocynthis* and stem-bark of *E. suaveolens* were collected, identified and authenticated at the herbarium unit of the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria. Fruits of *C. colocynthis* were blended with a mechanical grinder and dissolved in distilled water, filtrate was collected, and freeze-dried. *E. suaveolens* stem-barks were air dried at room temperature, ground to a fine powder and extracted with methanol.The mixture was filtered and filtrate was evaporated to dryness. The extracts were kept in McCartney's bottles and stored in the desiccator for further analysis.

Collection and Preparation of Test Organisms

Bacterial and fungal isolates were obtained from the stock of Microbiology laboratory of Adekunle Ajasin University, Akungba- Akoko. The test organisms used for the study were *E. coil, Pseudomonas. aeruginosa, S. aureus, B. cereus, Aspergillus niger, Aspergillus flavus, Trychophyton rubrum* and *Candida albican*. The isolates were sub-cultured from stock agar into nutrient agar broth and potato dextrose agar slant.

Antibacterial Screening

The antimicrobial screening of the crude extracts against selected bacterial isolates (*Escherichia coil, P. aeruginosa, S. aureus,* and *B. cereus*) was carried out using the agar well diffusion method (Balouiri *et al*., 2016). A stock concentration

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of 100 mg/ml was constituted by dissolving 1 g each, of the extracts in 2.5 ml of Dimethyl sulfoxide (DMSO) and diluted with 7.5 ml of sterile distilled water making 10 ml mixture (ratio 1:3). Concentrations of the extracts (50, 25, 12.5 and 6.125 mg/ml) were then prepared using dilution formula ($C_1V_1=C_2V_2$). An overnight culture of the bacteria was diluted to 0.5 Macfarland turbidity standard using sterile normal saline. The diluted broth culture (0.5 MacFarland turbidity standard) was inoculated on freshly Mueller Hinton agar plate by swabbing the entire agar surface with swab stick soaked with *inoculum*. The surface of the agar was allowed to dry and wells were bored on the agar using sterile cork borer. Fifty microlitre (50 µl) of the extracts and 10 mg/ml Oflaxacin (control) were dispensed into the wells using syringe. Plates were left on the workbench for 10 minutes to allow the diffusion of the extracts into the agar and were incubated at 37° C for 20 hours. The zones of inhibition diameter were measured and recorded in millimeter.

Antifungal screening

The antimicrobial screening of the crude extracts against the fungal isolates (*A. niger, A. flavus, T. rubrum*, and *C. albican*) was carried out using the agar well diffusion method (Balouiri *et al*., 2016). A stock concentration of 100 mg/ml was constituted by dissolving 1 g each, of the extracts in 2.5 ml of Dimethyl sulfoxide (DMSO) and diluted with 7.5 ml of sterile distilled water making 10 ml mixture (ratio 1:3). Concentrations of the extracts (50, 25, 12.5 and 6.125 mg/ml) were then prepared using dilution formula ($C_1V_1=C_2V_2$). The 72-hour fungal broth culture was diluted to 0.5 Macfarland turbidity standard using sterile normal saline. Potato dextrose agar (PDA) was prepared, sterilized and dispensed into sterile petri dish. The diluted broth culture (0.5 MacFarland turbidity standard) was inoculated on solidified PDA plates by swabbing the entire agar surface with swab stick soaked with the fungal inoculum. The surface of the agar was allowed to dry and wells were bored on the agar using sterile 6 mm cork borer. Fifty microlitre (50 µl) of the extracts and10 mg/ml fluconazole (control) were dispensed into the wells using micropipette. Plates were left on the workbench for 10 minutes to allow the diffusion of the extracts into the agar and were incubated at 25°C for 72 hours. The zones of inhibition diameter observed around the wells were measured with transparent ruler (mm).

Determination of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of the extracts b

The MIC of the extracts against the susceptible organisms was determined using the tube dilution method (Balouiri *et al*., 2016). A stock concentration of 200 mg/ml was constituted by dissolving 2 g each, of the extracts in 20 ml of Dimethyl sulfoxide (DMSO) diluted with sterile distilled water in ratio 1:3. Using Mueller Hinton broth as the diluents for bacteria and potato dextrose broth for fungi, 100, 50, 25 and 12.5 mg/ml were prepared from the stock solution in separate sterile test tubes. Then 1 ml of the standardized broth suspension (10^{-4}) was inoculated into each test tube and mixed thoroughly. The test tubes were then incubated at 37°C for 20 hours. The lowest concentration with no detectable growth was considered as the MIC.

The MBC of the extracts against the isolates was determined by subculturing the test dilutions with no visible growth onto a fresh nutrient agar plate and incubated further for 18 hours. The lowest dilution that yielded no bacterial growth solid medium was taken as minimum bactericidal concentration (MBC).

The minimum fungicidal concentration (MFC) of the extracts against the isolates was determined by subculturing the test dilutions with no visible growth onto a freshly potato dextrose agar plate and incubated further for 72 hours. The lowest dilution that yielded no bacterial growth solid medium was taken as MFC.

III. Results

The results of the antibacterial activity of aqueous fruit extract of *C. colocynthis* is presented in Table 1. This explains the zones of inhibition diameter (mm) exhibited by the extract against tested bacterial isolates (*E. coil, P. aeruginosa, S. aureus,* and *B. cereus*) at four concentrations. The zones of inhibition observed at 100 mg/ml ranged from 10 to 14 mm, 50 mg/ml was from 10 to 12 mm while 25 mg/ml ranged from 8 to 12 mm. It was observed that *C. colocynthis* extract did not inhibit *E. coil* at 25 mg/ml (0 mm). There was no activity shown at 12.5 mg/ml against all the bacteria tested. The control, Oflaxacin (10 mg/ml) showed zones of inhibition from 18 to 22 mm against the tested bacteria. Table 2 shows the inhibition diameter (mm) exhibited by MIC and MBC of *C. colocynthis* against the selected bacterial isolates. The zone of inhibition of MIC ranged from 25 to 50 mg/ml. The MBC concentration of *C. colocynthis* showed inhibition against the bacteria from 50 to 150 mg/ml. Furthermore,Table 3 presents the antifungi inhibitory effect of *C. colocynthis* extract against *A. flavus, C. albican, T. rubrum* and *A. niger* at various concentrations. The results at 100 mg/ml showed inhibition against all the tested fungi from 12 to 15 mm while 50 mg/ml was unable to inhibit the growth of *T. rubrum* (0 mm) and *A. niger* (0 mm) among the fungi tested. There was no activity/inhibition observed at 25 mg/ml (0 mm) and 12.5 mg/ml (0 mm). The control (Fluconazole; 10 mg/ml) exhibited higher zones of inhibition than the extract against all the fungi tested from the ranges of 17 to 19 mm. Table 4 presents the zones of inhibitory effects of MIC and MFC of *C. colocynthis* against *A. flavus, T. rubrum*, *C. albican* and *A. niger* (fungal isolates). The zones of inhibition of MIC ranged from 50 to 100 mg/ml. MFC concentration showed inhibition against all tested fungi (200 mg/ml) except for *T. rubrum* that had no data (ND).

Conversely, Table 5 explains the zones of inhibition diameter (mm) exhibited by *E. suaveolens* methanol extract against tested bacterial isolates (*E. coil, P. aeruginosa, S. aureus,* and *B. cereus*) at four concentrations. The zones of inhibition observed at 100 mg/ml ranged from 8 to 12 mm, 50 mg/ml could only inhibit *S. aureus,* and *B. cereus* at the ranges of 8 to 11. *E. suaveolens* extract showed no inhibition against all the tested isolates at 25 and 12.5 mg/ml. The control, Oflaxacin (10 mg/ml) showed zones of inhibition from 19 to

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22 mm against the tested bacteria.Table 6 shows the inhibition diameter (mm) exhibited by MIC and MBC of *E. suaveolens* against the bacterial isolates (*E. coil, P. aeruginosa* and *S. aureus, B. cereus)*. The zone of inhibition of MIC ranges from 50 to 100 mg/ml. For MBC, the plant showed inhibition against the bacteria ranging from 100 to 200 mg/ml. Table 8 presents inhibitory effect of *E. suaveolens* extract against *A. flavus, C. albican, T. rubrum* and *A. niger* (fungal isolates) at various concentrations. The results at 100 mg/ml showed inhibition against all the tested fungi from 10 to 12 mm except *T. rubrum* (0 mm), while 50 mg/ml was able to inhibit only *C. albican* (10 mm) among the fungi tested. There was no inhibition observed at 25 mg/ml (0 mm) and 12.5 mg/ml (0 mm).

Table 1: Antibacterial activity of *Citrullus colocynthis* extract

Microrganism	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	Control
Escherichia coil	13.0	10.0	0.0	0.0	22.0
Pseudomonas aeruginosa	12.0	12.0	10.0	0.0	18.0
Staphylococcus aureus	10.0	10.0	8.0	0.0	20.0
Bacillus cereus	14.0	12.0	12.0	0.0	20.0

Control; ofloxacin: 10 mg/ml, zones of inhibition measured in mm.

Table 2: Minimum Inhibition Concentration (MIC) and Minimum Bacterial Concentration (MFC) of *Citrullus colocynthis* extract against the bacterial isolates

Microorganism	MIC (mg/ml)	MBC (mg/ml)
Escherichia coil	50	100
Pseudomonas aeruginosa	25	100
Staphylococcus aureus	25	150
Bacillus cereus	25	50

Table 3: Antifungal activity of *Citrullus colocynthis* extract.

Microorganism	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	Control
Aspergillus niger	15.0	0.0	0.0	0.0	18.0
Aspergillus flavus	15.0	12.0	0.0	0.0	17.0
Trychophyton rubrum	12.0	0.0	0.0	0.0	19.0
Candida albican	14.0	12.0	0.0	0.0	18.0

Control; fluconazole; 10 mg/ml; Zone of inhibition measured in mm.

Table 4: Minimum Inhibition Concentration (MIC) and Minimum Fungal Concentration (MFC)of *Citrullus colocynthis* extract against the fungal isolates

Microorganism	MIC (mg/ml)	MFC (mg/ml)
Aspergillus niger	100	200
Aspergillus flavus	50	200
Trychophyton rubrum	100	ND
Candida albican	50	200

Table 5: Antibacterial activity of *Erythrophleum suaveolens* extract.

Microorganism	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	Control
Escherichia coil	9.0	0.0	0.0	0.0	22.0
Pseudomonas aeruginosa	8.0	0.0	0.0	0.0	19.0
Staphylococcus aureus	12.0	11.0	0.0	0.0	19.0
Bacillus cereis	10.0	8.0	0.0	0.0	20.0

Control; ofloxacin: 10 mg/ml; Zone inhibition measured in mm.

The control (Fluconazole; 10 mg/ml) exhibited higher zones of inhibition than the extract against all the fungi tested from the ranges of 17 to 20 mm. Table 9 presents the zones of inhibitory effects of MIC and MFC of *E. suaveolens* against *A. flavus, T. rubrum*, *C. albican* and *A. niger* (fungal isolates). The zones of inhibition at MIC ranges from 50 to 100 mg/ml. MFC showed inhibition against all tested fungi (200 mg/ml) except against *A. niger* and *T. rubrum* with no data (ND).

Table 6: Minimum Inhibition Concentration (MIC) and Minimum Bacterial Concentration (MBC) of *Erythrophleum suaveolens* extract against the bacterial isolates

Microorganism	MIC (mg/ml)	MBC (mg/ml)
Escherichia coil	100	200
Pseudomonas aeruginosa	100	150
Staphylococcus aureus	50	150
Bacillus cereus	50	100

Table 7: Antifungal activity of *Erythrophyleum suaveolens* extract

Microorganism	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	Control
Aspergillus niger	12.0	0.0	0.0	0.0	20.0
Aspergillus flavus	10.0	0.0	0.0	0.0	19.0
Trychophyton rubrum	0.0	0.0	0.0	0.0	19.0
Candida albican	10.0	10.0	0.0	0.0	17.0

Control; fluconazole: 10 mg/ml; Zone of inhibition measured in mm.

Table 8: Minimum Inhibition Concentration (MIC) and Minimum Fungal Concentration (MFC) of *Erythrophleum suaveolens* extract against the fungal isolates

Microorganism	MIC (mg/ml)	MFC (mg/ml)
Aspergillus niger	100	ND
Aspergillus flavus	100	200
Trychophyton rubrum	100	ND
Candida albican	50	200

IV. Discussion

The increasing trend of bacterial and fungal diseases in susceptible people, drug resistance (including both intrinsic and acquired) have resulted into an intense investigations into herbal medicines for their better efficacy and fewer side effects (Judaki *et al*., 2014). *C. colocynthis* and *E. suaveolens* have been used in traditional medicine for treatment of diseases. In this study, the antimicrobial effect of *C. colocynthis* and *E. suaveolens* extracts against pathogenic microorganisms (bacteria and fungi) was investigated.

The antimicrobial activity of *E. suaveolens* and *C. colocynthis* extracts against some clinical isolates showed that *C. colocynthis* extract gave highest concentration of inhibition against the fungal isolates *(A. flavus, A. niger, T. rubrum and C. albican*) at 100 mg/ml concentrations which ranges from 12 to 15 mm with highest inhibitory growth of 15 mm against *A. flavus* and *A. niger* and lowest inhibitory growth against *T. rubrum* (12 mm). The bacterial isolates for *C. colocynthis* revealed highest inhibitory growth against *B. cereus* (15 mm) and lowest inhibitory growth against *S. aureus* (10 mm). *E. suaveolens* extract at 100 mg/ml concentration ranges from 8 to 12 mm with highest zone of inhibitory diameter against *A. niger* (12 mm) and lowest against *T. rubrum* (0 mm) for fungi and highest inhibitory growth against *S. aureus* (12 mm) and lowest inhibitory growth against *E. coil* (9 mm). The minimum inhibition scores of *E. suaveolens* were higher than minimum inhibition records of *C. colocynthis* from 25 to 50 mg/ml (MIC) and 50 to 200 mg/ml (MBC and MFC)

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alongside some unrecorded data (ND). Lower MIC value of 10 mg/ml and MBC value of 50 mg/ml was reported by Tahmasebi *et al*. (2022). The differences in the zones of inhibition diameter revealed by the extract could be as a result of different extraction solvents. *E. suaveolens* extract showed inhibition ranging from 12 to 15 mm, MIC (50 to 100 mg/ml) and MFC (200 mg/ml) while control; fluconazole (10 mg/ml) ranges from 17 to 19 mg/ml.

The variations in the antimicrobial activity of the extracts may be as a result of difference in the quality and quantity of their bioactive components. The value of medicinal plants lies in phytochemical constituent that causes definite pharmacological action on the human body (Shah *et al*., 2015). The plants are the vital source of innumerable number of antimicrobial compounds. Several phytoconstituents like alkaloids, flavonoids, phenolics and polyphenols, tannins, terpenoids, sesquiterpenes etc., are effective antimicrobial substances against a wide range of microorganisms.

V. Conclusion

Citrullus colocynthis and *Erythrophleum suaveolens* extracts have demonstrated inhibitory and lethal effects on common pathogenic bacteria and fungi but *C. colocynthis* extract had higher activity against selected microorganisms than *E. suaveolens* extract. This study validates the use of these plants in traditional medicine. Furthermore, pure compounds or lead molecules responsible for their antimicrobial activity can be isolated and considered for the development of novel antimicrobial drugs.

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