Original Research Article

Comparative Investigation of the Antibacterial and Antifungal Potentials of the Extracts of Watermelon *(Citrullus lanatus)* Rind and Seed

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ABSTRACT

Aim: To investigate the antibacterial and antifungal potentials of the crude ethanol and aqueous extracts of watermelon (*Citrullus lanantus)* rind and seed.

Study design: The fruits were purchased from a local market and identified in the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture Umudike, Nigeria by a taxonomist. The rind and seed samples were extracted using ethanol (95%) and water and the crude extracts screened for antibacterial and antifungal potentials.

Place and Duration of Study: Department of Biochemistry, Michael Okpara University of Agriculture Umudike, Nigeria and National Root Crops Research Institute Umudike, Nigeria, between August, 2014 and November, 2014.

Methodology: The ethanol and aqueous extracts of watermelon (*Citrullus lanantus)* rind (EER and AER) and seed (EES and AES), each at a concentration of 100 mg/ml, were investigated by well-diffusion method for activity against ten pathogens.

Results: The EER exhibited the highest activity against the bacterium, *Esherichia coli* (6.0) and the fungus, *Candida albican* (4.0). The least activity was in the EES against the fungus, *Trichosporo bepelli* (0) followed by EER and AES against the bacterium, *Staphylococcus aureus* (1.0). For the bacterial strains, the highest mean activity (4.0) and mean susceptibility (4.50), respectively was in the EER and *Esherichia coli* whereas the least mean activity (3.0) and mean susceptibility (1.50), respectively was in AES and *Staphylococcus aureus*. For the fungal strains, the highest mean activity (2.5) and mean susceptibility (3.0), respectively was in the EER, AER, AES and *Candida albican* whereas the least mean activity (1.25) and mean susceptibility (1.25) was in EES and *Trichosporo bepelli*. The activity against the studied pathogens however was higher in the EE than in the AE of the rind and seed, suggesting the preference to the EE for higher pharmacologic activity.

Conclusion: The extracts (EER, AER, EES and AES) exhibited activity against the tested strains, especially *Escherichia coli* and *Candida albicans*. The study underscores the overriding potency of EER against the bacterium, *Escherichia coli* and the fungus, *Candida albicans*, and the non-susceptibility of the fungus, *Trichosporo bepelli* to EES. Further works, however are needed to validate reliability and possible exploitation in nutraceutical formulations and in ethnomedication.

Keywords: Nutraceutical, pharmacologic, pathogens, bacterial strains, fungal strains

1. INTRODUCTION

The search for plants and plant parts with pharmacologic activity is warranted because of the increasing emergence of hitherto unknown drug resistance diseases [1,2,3] and the need for a scientific basis for ethnomedicinal use of plants and plant parts extracts [4,5]. The use of plant based drugs in traditional medicine within Africa and the world over has been reported [6]. In particular, the medicinal value of fruits associated with their natural bioactive phytochemical contents [7] tends to increase fruit consumption the world over. In Nigeria, the pulp of various fruits, but not the rind and seed, is usually consumed. This contributes to food wastes in the environment. A relevant case is watermelon (family cucurbitaceae and specie *Citrullus lanatus*), a major fruit widely distributed in the tropics that serves as a thirst-quencher

owing to its high (92 %) water content [8,9]. To prevent solid waste related hazards to the environment, effort should be made to increase the utilization of food wastes which requires studies on their properties and pharmacologic activities.

The use of water extract of watermelon seed in traditional herbal medicine to cure catarrhal infection, fever, disorders of the bowel and urinary passage have been reported [10]. Studies on watermelon fruits were reported, but mainly on the juice/pulp [11,12] and a little on the peel/rind [13,14]. These warranted this study aimed at comparing the antibacterial and antifungal potentials of the crude ethanol and aqueous extracts of watermelon (*Citrullus lanantus)* rind and seed against these bacterial strains *viz*: *Escherichia coli, Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Bacillus pumilus, Staphylococcus aureus* and fungal strains *viz: Candida albicans, Aspergillus niger, Penicillium chrysogenum and Trichosporo bepelli*.

2. MATERIAL AND METHODS

2.1. *Chemicals and reagents*

All solvent (ethanol) and other chemicals used, including those used in the preparation of reagents, were of analytical grade and products of reputable companies. This study was carried out between August and November, 2014.

2.2. *Test organisms*

The organisms used for the test (clinical isolates of the bacteria and fungi strains from patients attending Federal Medical Centre Umuahia) were bought from HOSLAB Company Umuahia, Abia State, Nigeria. These include six bacterial strains (*Esherichia coli, Kiebsilla pneumonae, Pseudomonas aeruginosa, Bacillus subtilis* and *Bacillus* pumilus) and four fungal strains (*Aspergillus niger, Candida albicans*, *Trichosporo bepelli* and *Penicillium Chrysogenum*).

2.3. *Collection and preparation of samples*

Watermelon fruits were bought from Onuimo market, in Imo State border/boundary with Abia State, Nigeria. It was identified as Charleston gray variety in the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture Umudike, Nigeria. The watermelon was thoroughly washed to remove sand particles after which it was sliced using a home choice European knife. The seeds were handpicked and washed off the pulp particles using clean water. The pulp was carefully scraped off to obtain the rind which was chopped into pieces with a chipping machine.

The rind chips and seeds were respectively weighed, using Satorious Digital Weighing Balance, Model BP210S, Germany. The rind (wet weight = 1900.7 g) and seed (wet weight = 1016.9 g) were separately spread on a foil and sundried for 13 days to obtain the corresponding dry weight for the rind (82.6 g) and seed (468.5 g). The respective dry weight samples were milled into powder using Arthur Thomas Laboratory Mill, Crypto model, USA, covered separately in a labeled white nylon and kept in the desiccators until used.

2.4. *Preparation of ethanol extract*

To 10 g each of ground *Citrullus lanatus* seed and rind in a 250 ml conical flask were added 200 ml of ethanol and the content allowed to settle for 24 hrs. The filtrate of the extracts was obtained by separation of the suspension with a filter paper (Whatman filter paper No 1). The ethanol extracts were allowed to evaporate and then stored in an airtight conical flask until used.

2.5. *Preparation of aqueous extract*

The aqueous extracts of C*itrullus lanatus* samples were prepared by squeezing the sand-free specimen in triple distilled water. The resultant solution was filtered and dialyzed by using sigma dialysis membrane-500 (average flat width, 24.26 mm; average diameter, 14.3 mm, and approximate capacity, 1.61 $mlcm^{-1}$) against D-glucose to remove the excess water. The supernatant so obtained was lyophilized (Labcono Freeze Dry System) and stored at 4 ^{o}C in a refrigerator until used.

2.6. *Preparation of media*

7 gram each of nutrient agar and potato dextrose agar was weighed out in a beaker and dissolved in 250 ml of distilled water. The respective solutions were separately stirred, covered with a foil, autoclaved for 3 hours at 212 ^{o}C, allowed to cool in the autoclave (after reducing the temperature to 0 ^{o}C) and separately poured into the Petri dishes with the different bacteria and fungi strains.

2.7. *Antibacterial activity*

Petri dishes with nutrient agar were inoculated with six different species of bacteria. *Citrullus* species extraction was sterilized by passing each through a 0.22: m Millipore GV filter paper (Millipore USA). Round paper dishes with a radius of 0.8 cm were dipped into each extract (at a concentration of 100 mg/ml) and placed in the center of the inoculated petri-dishes. Bacterial colonies were allowed to grow overnight (24 hours) at 37 ^{o} C, and the inhibition zone (activity) around the disc measured.

2.8. *Antifungal activity*

Petri dishes with potato dextrose agar were inoculated with three different species of fungus. *Citrullus* species extracts were sterilized by passing each through a 0.22: m Millipore GV filter paper (Millipore USA). Round paper dishes with a radius of 0.8 cm were dipped into each extract (at a concentration of 100 mg/ml) and placed in the center of the inoculated petri-dishes. Fungal colonies were allowed to grow 48 hours at 28 ^{o} C and the inhibition zone (activity) around the disc measured.

2.9. *Data*

Data were presented simple mean obtained from duplicate measurement of each sample.

3. RESULTS AND DISCUSSION

The pharmacologic activities of the crude ethanol extracts (EE) and aqueous extracts (AE) of watermelon (*Citrullus lanantus)* rind (EER and AER) and seed (EES and AES) at a concentration of 100 mg/ml were investigated by well-diffusion method against ten pathogens (six bacterial and four fungal strains). The EER exhibited the highest activity/inhibition zone diameter (mm) against the bacterium, *Esherichia coli* (6.0) and the fungus, *Candida albican* (4.0). The least activity was in the EES against the fungus, *Trichosporo bepelli* (0) followed by EER and AES against the bacterium, *Staphylococcus aureus* (1.0) (Tables 1 and 2).

Table 1: Activity/inhibition zone diameter (mm) of the samples extracts against the bacterial strains: *Esherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Bacillus subtilis*

	INHIBITION ZONE DIAMETER, IZD (MM) AT A CONCENTRATION OF 100 MG/ML				
BACTERIAL STRAIN	**ETHANOL EXTRACT OF RIND (EER)**	**ETHANOL EXTRACT OF SEED (EES)**	**AQUEOUS EXTRACT OF RIND (AER)**	**AQUEOUS EXTRACT OF SEED (AES)**	**MEAN SUSCEPTIBILITY TO EXTRACTS**
ESHERICHIA COLI	6.0	4.0	4.0	4.0	4.50
KLEBSIELLA PNEUMONIA	4.0	4.0	5.0	3.0	4.00
PSEUDOMONAS AERUGINOSA	5.0	3.0	4.0	5.0	4.25
BACILLUS SUBTILIS	4.0	4.0	3.0	3.0	3.50
MEAN ACTIVITY (INCLUDING VALUES FOR *B. PUMILUS* AND *S. AUREUS*)	4.0	3.3	3.3	3.0	

Simple mean of duplicate measurements.

Table 2: Activity/inhibition zone diameter (mm) of the samples extracts against the fungal strains: *Candida albican, Aspergillus niger*, *Penicillium chrysogenum* and *Trichosporo bepelli*

	INHIBITION ZONE DIAMETER, IZD (MM) AT A CONCENTRATION OF 100 MG/ML)				
FUNGAL STRAIN	**ETHANOL EXTRACT OF RIND (EER)**	**ETHANOL EXTRACT OF SEED (EES)**	**AQUEOUS EXTRACT OF RIND (AER)**	**AQUEOUS EXTRACT OF SEED (AES)**	**MEAN SUSCEPTIBILITY TO EXTRACTS**
CANDIDA ALBICAN	4.0	2.0	3.0	3.0	3.0
ASPERGILLUS NIGER	3.0	1.0	2.0	2.0	2.0
PENICILLIUM CHRYSOGENUM	2.9	2.0	3.0	3.0	2.5
TRICHOSPORO BEPELLI	1.0	0.0	2.0	2.0	1.25
MEAN ACTIVITY	2.5	1.25	2.5	2.5	

Simple mean of duplicate measurements.

For the bacterial strains, the highest mean activity (4.0) and mean susceptibility (4.50), respectively were in the EER and *Esherichia coli* whereas the least mean activity (3.0) and mean susceptibility (1.50) were in AES and *Staphylococcus aureus* (Table 1, Figures 1 and 2). The highest mean activity (2.5) and mean susceptibility (3.0), respectively for the fungal strains were in the EER, AER, AES and *Candida albican* whereas the least mean activity (1.25) and mean susceptibility (1.25) were in EES and *Trichosporo bepelli* (Table 2).

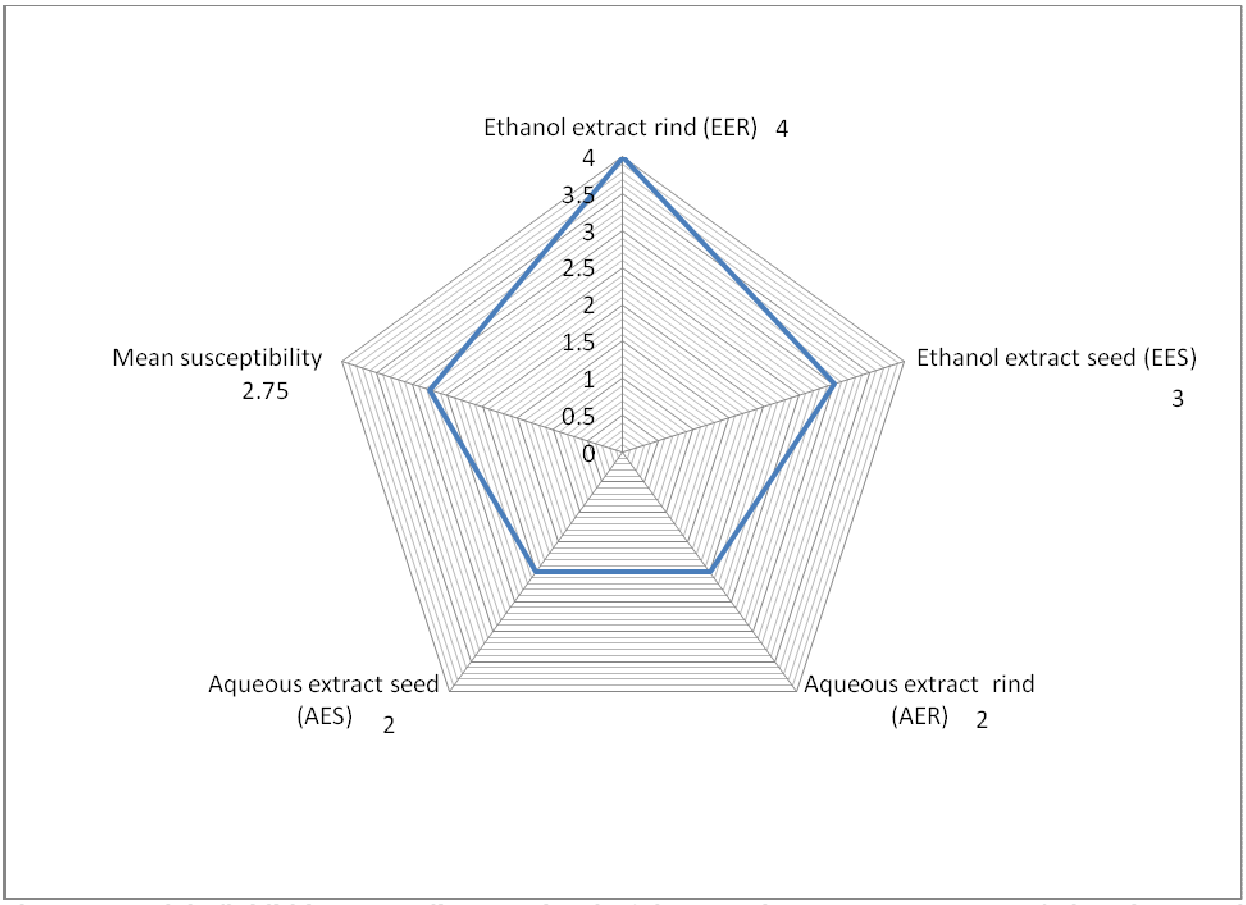

Figure 1: Activity/inhibition zone diameter (mm) of the samples extracts at 100 mg/ml against *Bacillus pumilus*

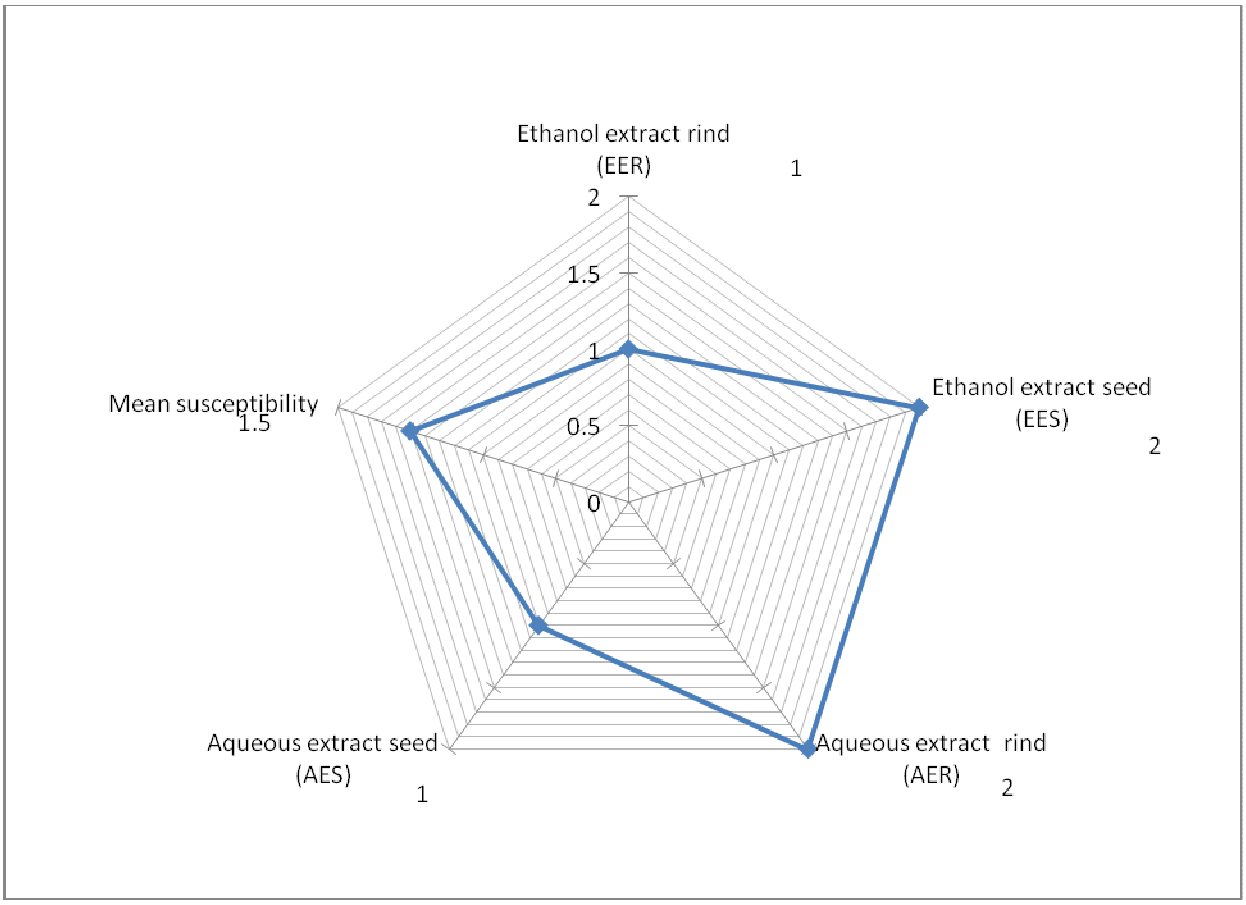

Figure 2: Activity/inhibition zone diameter (mm) of the samples extracts at 100 mg/ml against *Staphylococcus aureus*

Generally, the results revealed that the extracts were effective against the tested pathogens. The ethanol extracts of the samples however showed high antibacterial activities than the aqueous extracts, in consonance with the result of [15]. In particular, the activity of the extracts against *P. aeroginosa* were higher than that reported in Obasi *et al. [5]* for extracts and fractions of *Jatropha curcas* stem bark, although the chemical composition and the concentration of the extracts and fractions are different. The activities of the extracts against *Bacillus subtilis* and *Staphylococcus aureus* were lower than what were reported by Obasi *et al. [2]* for methanol and ethyl acetate extracts of *Samanea saman* pods at 100 mg/ml and even 50 mg/ml. The EER exhibited the highest activity against the bacterium, *Esherichia coli* (6.0) and the fungus, *Candida albican* (4.0), indicating the overriding efficacy of EER against the pathogens. Antibacterial and antifungal activities were attributed to the presence of antinutrients [16,17], warranting further studies on the antinutrient compositions of the watermelon rind and seed.

For the bacterial strains, the highest mean activity (4.0) and mean susceptibility (4.50), respectively were in the EER and *Esherichia coli* whereas the least mean activity (3.0) and mean susceptibility (1.50) were recorded in AES and *Staphylococcus aureus*. This suggests the higher potency of EER over others (EES, AES and AER) against ailments caused by bacteria, notably *Esherichia coli.* For the fungal strains, the highest mean activity (2.5) and mean susceptibility (3.0), respectively were in the EER, AER, AES and *Candida albican*, suggesting the potency of these extracts of watermelon against *Candida albican* and possibly diseases caused by *Candida albican*. The least mean activity against (1.25) and mean susceptibility to (1.25) the fungal strains were recorded in EES and *Trichosporo bepelli*. Furthermore, the least activity was recorded in EES against *Trichosporo bepelli* (0). These imply that the extract (EES) could neither combat *Trichosporo bepelli* nor serve as broad spectrum antibiotic. The activities were higher in the EE than in the AE of the rind and seed, suggesting the preference to the EE for higher pharmacologic activity. The extracts of the rind has higher antibacterial and antifungal activities than that of the seed, suggesting that the rind could be a better source for pharmacologic agents against the studied pathogens.

4. CONCLUSION

The extracts (EER, AER, EES and AES) exhibited activity against the tested strains, especially *Escherichia coli* and *Candida albicans*. The study underscores the overriding potency of EER against the bacterium, *Escherichia coli* and the fungus, *Candida albicans*, and the non-susceptibility of the fungus, *Trichosporo bepelli* to EES. Further works, however

are needed to validate reliability and possible exploitation in nutraceutical formulations and in ethnomedication The exact volume of extract used to incubate the pathogens was not recorded but presumed the volume that could be absorbed by the described disc when dipped into and immediately removed from the extract. This is a noted shortcoming that could be taken care of in further similar studies.

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