

Full Length Research Paper

Antibacterial activity of watermelon (*Citrullus lanatus*) seed against selected microorganisms

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This study was aimed at evaluating the effect of extraction methods on the antibacterial activity of *Citrullus lanatus* seed extract. *C. lanatus* (watermelon) is a popular fruit consumed all over the world. Three solvents were used for the extraction process: chloroform, methanol and distilled water while two extraction conditions- cold extraction and Soxhlet extraction (coded as hot in this study) were employed. Antibacterial activity of the seed extracts was determined by agar well diffusion method. The seed extracts were tested against clinical isolates including *Staphylococcus* sp., *Escherichia coli*, *Proteus* sp., *Klebsiella* sp. and a type *Pseudomonas aeruginosa* (ATCC 27853). It was observed that the cold methanol extracts had the highest antibacterial effect on *Staphylococcus* sp. followed by hot methanol extract while cold chloroform extract showed no antibacterial activity. In the presence of *P. aeruginosa* only the hot methanol and chloroform extracts showed significant antibacterial potentials ($p \leq 0.05$). Also, saponins which have been implicated in antimicrobial activity were found to be present in moderate and high concentrations in the hot and cold methanol extracts respectively. Results of this study reveal that the kind of solvent employed as well as the conditions for extraction (cold maceration and Soxhlet extraction) influenced the efficacy of the extract against specific test organisms. Furthermore, the presence of saponins may have influence the relatively high zone of inhibition recorded with cold and hot methanol extracts against some of the test organisms.

Key words: Watermelon seed, antibacterial, Soxhlet extraction, cold maceration, solvents.

INTRODUCTION

Citrullus lanatus commonly called watermelon is a popular fruit in many parts of the world and it is notable for its high water content and attractive look. The fruit comes in various shapes, sizes and rind pattern (Wehner, 2008). Although the seed of watermelon is often discarded as

waste; it contains various amounts of carbohydrate, phenol, flavonoids, protein, fibre, phosphorus and iron (Varghese et al., 2013). Proximate analysis of the seed as reported by Oyeleke et al. (2012) revealed very high fat content (47.9%) followed by protein (27.4%) and

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carbohydrate (9.9%). Traditionally, the seed of *C. lanatus* is said to be medicinal because it can relieve inflammation/irritation; causes increased passing of urine and gives tonic effects (Okunrobo et al., 2012; Varghese et al., 2013).

Extraction of medicinal plants is carried out using various methods including maceration, infusion, decoction, percolation, hot continuous extraction (Soxhlet extraction), thermal desorption, surfactant mediated extraction, counter-current extraction, accelerated solvent extraction, pressurized liquid extraction amongst others. Advanced extraction methods include extraction, ultrasonication assisted extraction, supercritical fluid extraction, microwave-assisted extraction and supercritical fluid extraction (Gupta et al., 2012; Handa, 2008). Most of the information available on the antibacterial activity of *C. lanatus* seed have made it clear that cold maceration is popularly used for extraction of *C. lanatus* plant parts (Okunrobo et al., 2012; Omigie and Agoreyo, 2014; Oseni and Okoye, 2013); and in few cases Soxhlet extraction (Meena and Patni, 2008; Oyeleke et al., 2012). This has brought about the need to compare extraction methods in order to determine which method will yield the highest antibacterial effect on the organisms. The aim of this study was to examine the effect of extraction solvents and extraction conditions on the antibacterial potential of the seed extracts of *C. lanatus*.

MATERIALS AND METHODS

Collection and preparation of seed material

Twenty five watermelon fruits were purchased from Omu-aran market, Kwara State, Nigeria. The fruits were washed and cut open to obtain the seeds. The seeds obtained were washed and air-dried for two days; then pulverized using mortar and pestle under aseptic conditions and ground to powder using a blender (Waring commercial blender, Model No. HGB2WTS3). Powdered seed material were then weighed and kept in air-tight containers until further usage.

Preparation of crude extracts

Cold extraction by maceration and Soxhlet extraction were carried out on the seed material using three solvents- chloroform, methanol and distilled water- in order of increasing polarity.

Cold extraction: The powdered seed material was subjected to successive solvent extraction using chloroform, methanol and distilled water in the increasing order of polarity. A total of 50 g each of dried seed powder was extracted first in 150 ml of chloroform in a conical flask and placed in an orbital shaker for 72 h at 90 rpm. This was done in triplicate. The extracts obtained were filtered using Whatman filter paper and were evaporated to dryness using a rotary evaporator and water bath at 40-50°C then stored at 0-4°C in air-tight containers for further use (Varghese et al., 2013). The residue was allowed to air-dry and subsequently extracted with 150 ml of methanol following the same procedure. Lastly, the air-dried residues were extracted with 150 ml of distilled water also following the method but for 24 h.

Soxhlet extraction: The powdered seed material was also subjected to successive solvent extraction using chloroform, methanol and distilled water in the increasing order of polarity. The Soxhlet apparatus was used in this case. A total of 50 g each of dried seed powder was extracted in three thimbles (triplicate) of the Soxhlet apparatus, first in 150 ml each of chloroform and allowed to stand for 6 h. The extracts obtained were evaporated to dryness using a rotary evaporator and water bath at 40-50°C, and stored at 0-4°C in air-tight container for further use (Varghese et al., 2013). The residue was allowed to air-dry and extracted with 150 ml of methanol following the same method. Distilled water was not used for Soxhlet extraction due to the challenges encountered. All extracts were evaporated to dryness using a water bath.

Phytochemical screening

Qualitative phytochemical screening of the various extracts was carried out according to standard procedure (Trease and Evans, 1989; Evans et al., 2002) to ascertain the qualitative composition of the seed. Phytochemicals screened include alkaloids, phenols/tannins, saponins, steroids, flavonoids, reducing sugars and lipids.

Dilution of extract

In 1 ml of each solvent used except chloroform, 0.2 g (200 mg) of the corresponding extract was re-dissolved. The chloroform extract on the other hand was re-dissolved in a mixture of petroleum ether and methanol (8:2 v/v). Ciprofloxacin was used as positive control. 1000 mg of Ciprofloxacin was dissolved in 5 ml of distilled water.

Preparation of test specimens

Clinical isolates of *Staphylococcus* sp., *Escherichia coli*, *Proteus* sp., and *Klebsiella* sp. were obtained from the University of Ilorin Teaching Hospital, Ilorin, Kwara State, as well as a type strain of *P. aeruginosa* (ATCC 27853). The test organisms were sub-cultured and incubated at 37°C for 24 h on nutrient agar slant medium and were stored 0-4°C till use. They were then further sub-cultured in nutrient broth at 37°C for 24 h.

In-vitro antibacterial activity (agar well diffusion method)

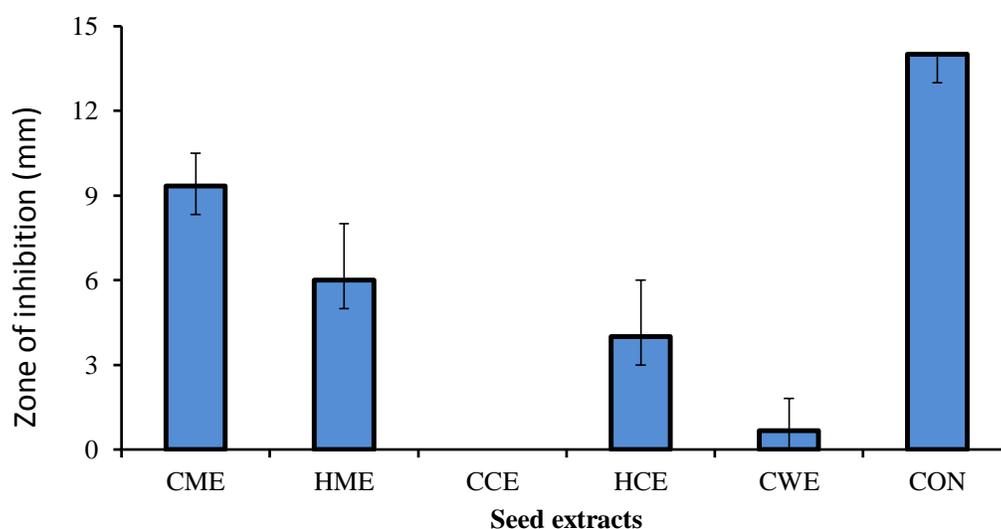
Antibacterial activity of watermelon seed extracts was carried out using the agar well diffusion method as reported by Hassan et al. (2011), with some modifications. Mueller Hinton agar (LAB039, LabM Limited, UK) was prepared according to the manufacturer's direction. From each standard bacterial stock suspension [*Staphylococcus* sp. (1.5×10^5 CFU/ml); *E. coli* (2.0×10^5 CFU/ml); *P. aeruginosa* (3.4×10^5 CFU/ml)], 1 ml of the broth culture of the respective bacteria was mixed thoroughly with 20 ml of sterile molten Mueller Hinton agar (45-50°C) before pouring into sterile petri dishes and left to solidify. Furthermore, a sterile cork-borer of diameter 6.0 mm was used to bore wells in each agar plate and 50 µl of each extract was introduced into the different wells. The plates were allowed to stand for 6 h after which they were incubated for 18 to 24 h at 37°C. After incubation, the zone of inhibition for each extract and the control was measured using a meter rule and recorded.

Statistical analyses were carried out using the SPSS statistical software. The comparison of means was done using the One-Way Analysis of Variance (ANOVA). All experimental setups were in triplicate.

Table 1. Qualitative phytochemical analysis of *Citrullus lanatus* seed extracts

Phytochemicals	CCE	HCE	CME	HME	CWE
Saponins	-	-	+++	++	+
Phenols/Tannin	-	-	++	+++	-
Alkaloids	-	-	++	++	++
Steroids	+++	+++	+	++	-
Flavonoids	+++	++	+++	+++	-
Reducing sugars	++	++	+++	+++	-
Lipids	++	++	+	+	+

CCE= Cold chloroform extract; HCE= hot chloroform extract; CME= cold methanol extract; HME= hot methanol extract; CWE= cold water extract; +++= High concentration; ++= moderate concentration; += low concentration; - = absent. The solvents were evaporated to dryness.

**Figure 1.** Antibacterial potential of the different seed extracts of the watermelon on *Staphylococcus sp.*

RESULTS

Qualitative phytochemical analysis

The various extracts of watermelon seeds were tested for different phyto-constituents including alkaloids, phenols/tannins, saponins, steroids, flavonoids, reducing sugars and lipids using standard procedures and the results are shown in Table 1. The result reveals that the cold chloroform and hot chloroform extracts (CCE and HCE) had the same phyto-constituents; this was also the case with the cold and hot methanolic extracts (CME and HME). In addition, the methanol extracts (CME and HME) were positive for all phytochemicals screened.

Antimicrobial screening

As shown in Figure 1, the highest zone of inhibition of 9.3

mm was recorded with the cold methanol extract (CME) followed by hot methanol extract (HME) which had a zone of inhibition of 6.00 mm when administered against *Staphylococcus sp.*

Among the different extracts used for investigation, the CME and HME extracts were observed to show significantly high zones of inhibition against *Staphylococcus sp.*, when compared with the other extracts ($p \leq 0.05$). Significantly low zones of inhibition were observed with the CCE and the CWE in the presence of the *Staphylococcus sp.* ($p \leq 0.05$).

When *E. coli* was used as the test bacteria, the antibacterial activity of the various extracts against *E. coli* was generally low apart from the control (Figure 2). CWE recorded a zone of inhibition of 6.0 mm while HCE had a zone of inhibition of 5.33 mm. When compared to the control antibiotic, none of the extracts used for investigation was observed to significantly inhibit the growth of *Escherichia coli*. The zones of inhibitions

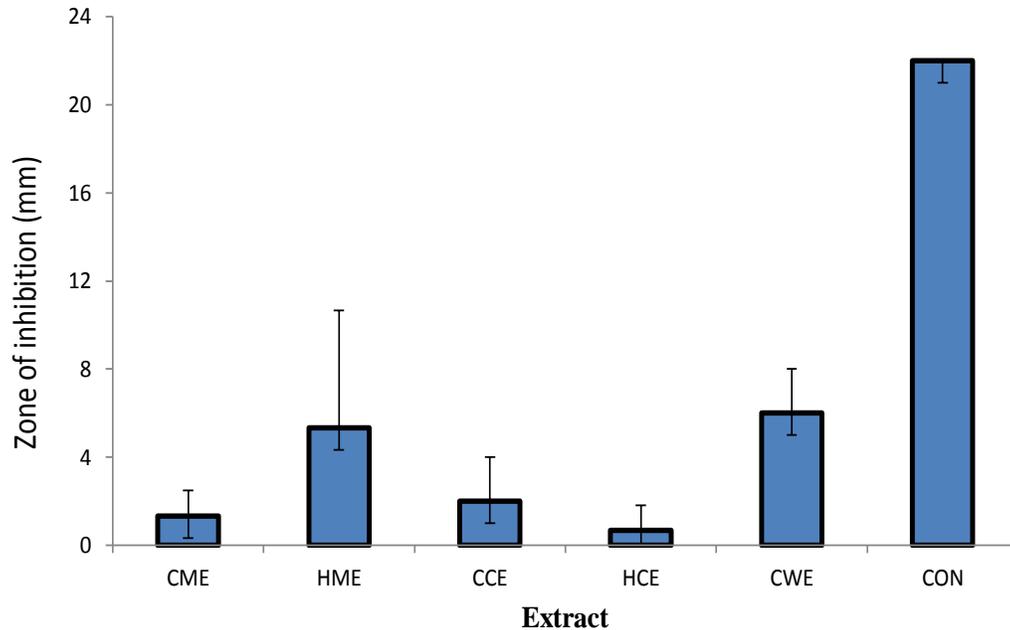


Figure 2. Antibacterial potential of the different seed extracts of the watermelon on *Escherichia coli*.

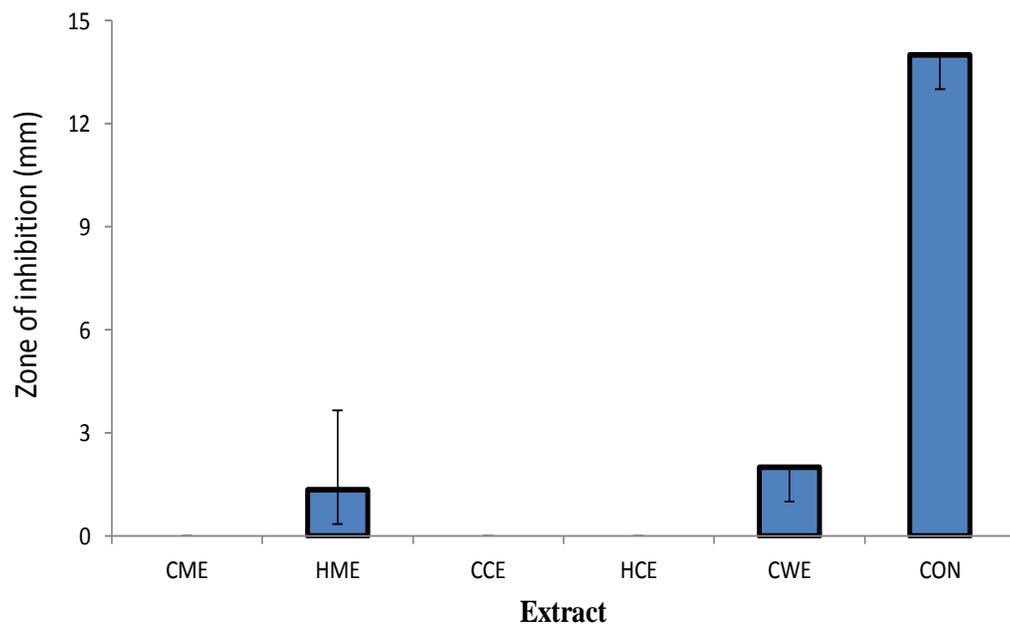


Figure 3. Antibacterial potential of the different seed extracts of the watermelon on *Proteus sp.*

observed for 'HME' and 'CWE' were however observed to be significantly higher than those of the other extracts ($p \leq 0.05$).

Figure 3 shows that CME, HCE and CCE extracts did not show any zone of inhibition when tested against *Proteus sp.* while CWE and HME recorded zones of inhibition of 2 and 1.33 mm, respectively.

In the presence of *Proteus sp.*, the zones of inhibition showed by the different extracts were not observed to be significantly different from one another. A significantly high zone of inhibition was however observed for the control antibiotic ($p \leq 0.05$).

When tested against *Klebsiella sp.*, extracts HCE and CWE recorded similar zone of inhibition of 2.67 mm,

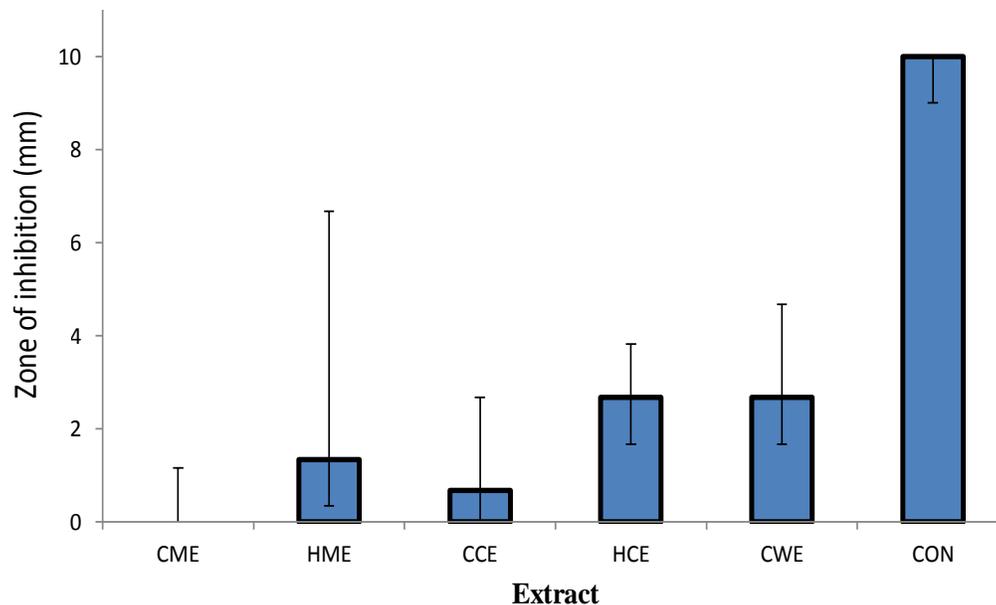


Figure 4. Antibacterial potential of the different seed extracts of the watermelon on *Klebsiella* sp.

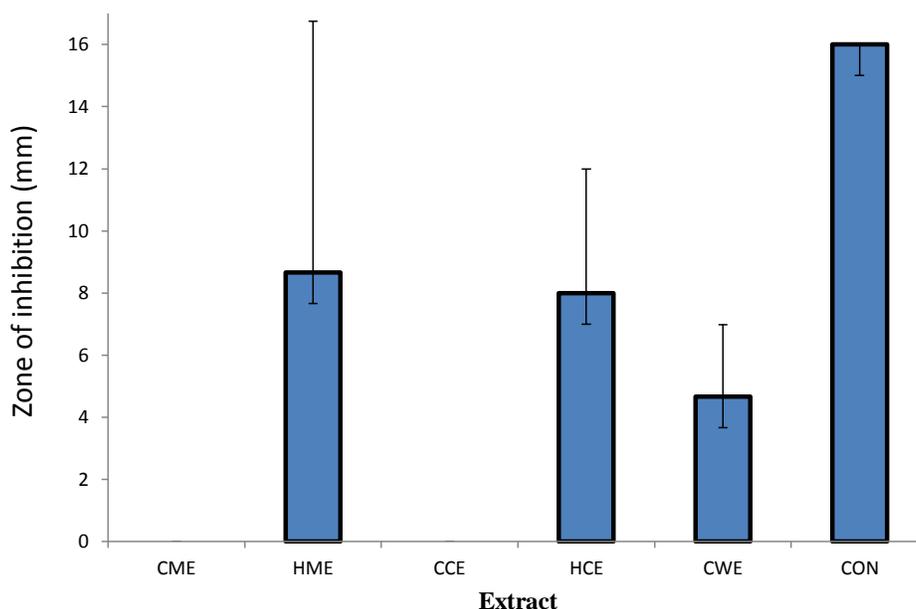


Figure 5. Antibacterial potential of the different seed extracts of the watermelon on *Pseudomonas aeruginosa*.

CME showed no zone of inhibition while the control had a zone of inhibition of 10 mm (Figure 4). As was observed in the presence of the *Proteus* sp., although the zone of inhibition observed for the control antibiotic was observed to be significantly higher than all the extracts used for the investigation, no significant differences in zones of inhibition were observed among the extracts in the presence of the *Klebsiella* sp. ($p \leq 0.05$).

In the case of *P. aeruginosa*, antibacterial activity was highest with HME (8.67 mm) followed closely by HCE (8.0 mm) while CME and CCE showed no zone of inhibition (Figure 5).

Although remarkably high zones of inhibition were observed in the presence of the *Pseudomonas aeruginosa* for the HME, HCE and CWE extracts, only the 'HME' and 'HCE' were observed to be significantly different ($p \leq 0.05$).

DISCUSSION

The presence of saponins in high concentration in CME may have resulted in the extract having the highest zone of inhibition recorded during this study. In the same vein, the concentration of saponins in HME and CWE were moderate and low respectively and this was evident in their activity against *Staphylococcus* sp. being the only Gram positive organism studied. Saponins have been reported to have antibacterial effect against Gram positive bacteria but not against Gram negative organisms (Soetan et al. 2006). The antibacterial activity of CME and HME against the *Staphylococcus* sp. in the present study can be ascribed to the presence of saponins, this was corroborated by the report of Soetan et al. (2006) and Thirunavukkarasu et al. (2010) that crude saponin extracts of *Sorghum bicolor* L. Moench and pure saponin fraction of the leaves of *Solanum trilobatum* Linn were active against *S. aureus*.

In the present study significant antibacterial effect was observed against *Staphylococcus* sp. (in the case of cold methanol and hot chloroform extracts) and *P. aeruginosa* (when the hot methanol and hot chloroform extracts were tested). This aligns with the report of Adewuyi et al. (2013) who evaluated the antibacterial activities of nonionic and anionic surfactants from *Citrullus lanatus* seed oil; they reported that *C. lanatus* seed oil did not show any inhibition against the tested organisms while the biosurfactants had activity against the growth of the test organisms, *P. aeruginosa*, *S. aureus*, *K. pneumonia* and *E. coli*. This study revealed that the *Klebsiella pneumonia* was not susceptible ($p \leq 0.05$) to any of the extracts while the *Pseudomonas aeruginosa* was susceptible to hot methanol (HME) and chloroform (HCE) extract; but Braide et al. (2012) also determined the antibacterial activity of aqueous, methanol and ethanol extracts of *C. lanatus* seed on five bacteria and found *K. pneumonia* and *P. aeruginosa* to be susceptible to all extracts. This might be as a result of the condition under which the extraction was carried out in this study; Soxhlet apparatus while that reported by Braide et al. (2012) was carried out without the introduction of any form of heat.

Conclusion

The antibacterial effects of *C. lanatus* seed extracts against the selected bacteria suggests that extracts obtained by cold maceration, Soxhlet extraction, as well as using methanol and chloroform have potential as antibacterial agents especially against *Staphylococcus* sp. and *P. aeruginosa*. Further isolation of saponins from seed and testing it against more Gram positive bacteria

will be necessary to confirm that saponins are responsible for the antibacterial activity observed.

Conflict of interests

The authors did not declare any conflict of interest.

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