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Preliminary Phytochemical Investigation and Antibacterial Activity of *Citrulus lanatus'* Rind

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Abstract

The emergence of multidrug resistant bacteria has spurred diverse search for new antimicrobial agents. Agricultural wastes like the water melon rind would be a good source of such natural product since they do not threaten food security.Fresh and oven-dried watermelon rinds were serially extracted with n-hexane and methanol, using maceration and soxhlet extraction methods respectively. These extracts were subjected to antibacterial assay using the agar well diffusion method and the most active of the extracts was subjected to column chromatography (CC). Antibacterial activity was determined for each fraction combination. Six extracts were obtained and the phytochemical screening revealed the presence of phenols/tannins, saponins, steroids, cardiac glycosides, flavonoids and lipids. The oven-dried methanolic extract (MO) showed the highest zone of inhibition (ZOI) of 8 mm and was further subjected to column chromatography. The CC yielded some fractions which showed higher zone of inhibition against Proteus sp., Escherichia coli and Pseudomonas aeruginosa (ATCC 27853) while all the fractions showed lower ZOI against Staphylococcus sp. Therefore, watermelon rind could be an abundant source of antimicrobial extracts whose antibacterial activity could be enhanced by employing chromatographic fractionation.

Keywords: Waste; Water melon; Phytochemicals; Antimicrobial; Chromatography

1.0 Introduction

Watermelon (*Citrullus lanatus*) is a member of the Cucurbitaceae family (Dane and Lu, 2007). It is a popular fruit from which a huge amount of waste is generated on a consistent basis. Among several efforts which have been directed towards the seed of this plant, Adelani-Akande *et al.* (2015) recently reported the antimicrobial activity of watermelon seed against selected microorganisms. Furthermore, the rind of this plant has been reported to be a rich source of natural citrulline, a non-essential amino acid, which is more abundant in the rind than the flesh (Rimando and Perkins, 2005). The quantity of rind discarded as waste from water melon fruit is enormous and discovering new uses for these rinds (wastes) is in line with current efforts at sustainable development involving 'waste to wealth' (Oluyori *et al.,* 2015; Oluyori *et al*., 2016).

Specifically, the effect of extraction solvent and extraction temperature on the antibacterial potential of water melon seed extracts has been investigated. However, water melon rind has not been studied in this regard. This work is therefore aimed at examining the effect of extraction solvent, extraction temperature and column chromatographic separation on the phytochemical constitution and antibacterial potential of *Citrullus lanatus* rind.

2.0 Materials and Methods

2.1 Sample Preparation

12 fruits of watermelon were collected from the market in Omu-Aran, Kwara State. The endocarp and mesocarp of the watermelon fruits were carefully removed with a knife and the epicarp (the rind), was cut into small cubes as shown in Figure 1.

Figure 1: Cubes samples of watermelon rind

2.2 Extraction

Maceration was used to effect extraction at room temperature while soxhlet extraction was adopted for high-temperature extraction.

2.2.1 Cold extraction:

1.5 kg of the freshly cut samples was successively macerated using n-hexane, methanol and water. The extraction time with each solvent was one (1) week with daily manual agitation/swirling after which the solvent with the extract was filtered and the filtrate concentrated to obtain the crude extract.

2.2.2 Hot extraction:

The oven dried samples were ground to powder using a blender/grinder and 7.5 g of the pulverised rind was successively extracted with n-hexane and methanol using the soxhlet apparatus. Each time, the extraction lasted for six (6) hours.

2.3 Phytochemical Screening

The phytochemical screening of the various extracts was carried out according standard procedure (Harborne, 1993; Thamaraiselvi and Jayanthi, 2012; Oluyori and Olatunji, 2016) to ascertain the phytochemical composition of the extracts. The extracts were qualitatively screened for the presence of the following phytochemicals: alkaloids, phenols/tannins, saponins, steroids, cardiac glycosides, flavonoids, and lipids.

2.4 Antimicrobial Activity of Watermelon Rind Crude Extract

Organisms used for testing the antimicrobial activity of extracts were obtained from the Microbiology laboratory and courtesy Dr. O.B. Akpor of the Microbiology unit, Landmark University, Omu-Aran, Nigeria. The organisms were sub-cultured on Nutrient agar and stored in the refrigerator until further use. The extracts were tested against the following clinical isolates: *Staphylococcus* sp., *Escherichia coli*, *Proteus* sp., *Klebsiella* sp. and *Pseudomonas aeruginosa* (ATCC 27853). Bacteria were grown in nutrient broth and later diluted to 0.5 mc Farland standard. Agar well diffusion method was used to determine the antibacterial activity of the watermelon rind extracts as described by Hassan *et al*. (2011) with slight modifications. Culture plates were incubated and the diameter (mm) of the zone of inhibition (ZOI) was measured with a ruler and mean diameter was recorded.

2.5 Column Chromatography

Silica gel Column Chromatography (CC) was used to fractionate the most promising crude extract. The column was packed wet with silica gel (100 – 200 mesh size) according to the normal procedure and Thin Layer Chromatography (TLC) was used to monitor the CC.

3.0 Results and Discussion

3.1 Phytochemical Screening

The results of the preliminary phytochemical screening carried out on water melon extracts is shown in Table 1. It confirms the presence of saponins, flavonoids, steroids, tannins, cardiac glycosides and lipids. The presence of saponins in the oven dried extracts shows that oven drying and soxhlet extraction obviously increased the extraction yield of the saponins while the flavonoids were more soluble in methanol than in water. The observed results also suggest that the flavonoids might be thermo-labile since their presence seemed to reduce in the extracts obtained via soxhlet extraction. As expected, the steroids were more soluble in n-hexane while the cardiac glycoside seems to be abundant in all the extracts.

Table 1**:** Result of the Preliminary Phytochemical Screening

	Alkaloids	**Saponins**	**Flavonoids**	**Steroids**	**Tannins**	**Cardiac Glycosides**	**Lipids**
HC	-	-	-	+++	-	++	-
HO	-	+++	-	++	-	++	-
MC	-	-	+++	++	-	+++	+++
MO	-	++	+	-	-	+	+
WC	-	-	++	+	+	++	++
WO	-	+	-	-	-	++	-

Key: HC=Hexane/Cold, HO=Hexane/Ovendried, MC=Methanol/Cold, MO=Methanol/Ovendried,

WC=Water/Cold, WO=Water/Ovendried

+++= Significantly present; ++= Moderately present; += Slightly present; - = absent

3.2 Antimicrobial activity

The six extracts obtained as described above, were investigated for their anti-bacterial potential. Although the antibacterial activity was low, MO (soxhlet mediated oven dried methanolic extract) demonstrated the most promising activity (Table 2; Figure 2). Based on the phytochemical results in table 1, saponins which were present in MO but not detected in MC, are suspected to be responsible for the observed better activity in MO. This is in line with previously documented facts about saponins (Adelani-Akande *et al.*, 2015)

Table 2: Antimicrobial activity (Zones of Inhibition) of watermelon rind extracts

	Staphylococcus sp.	***Proteus sp.***	***E. coli***	***Pseudomonas aeruginosa***	***Klebsiella sp.***
MC	**6**	**0**	**0**	**0**	**3**
MO	**10**	**5**	**2**	**4**	**4**
WC	**0**	**0**	**0**	**0**	**2.5**
WO	**0**	**0**	**0**	**0**	**2.5**
CON	**14**	**13.5**	**21.5**	**15.5**	**10**

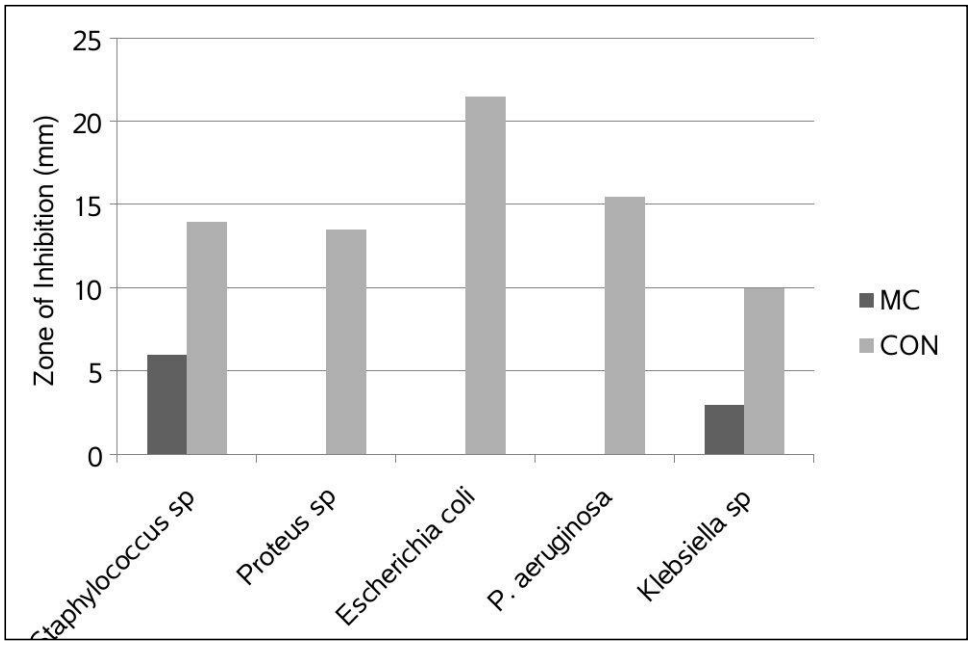


Figure 2a: Antibacterial potential of MC

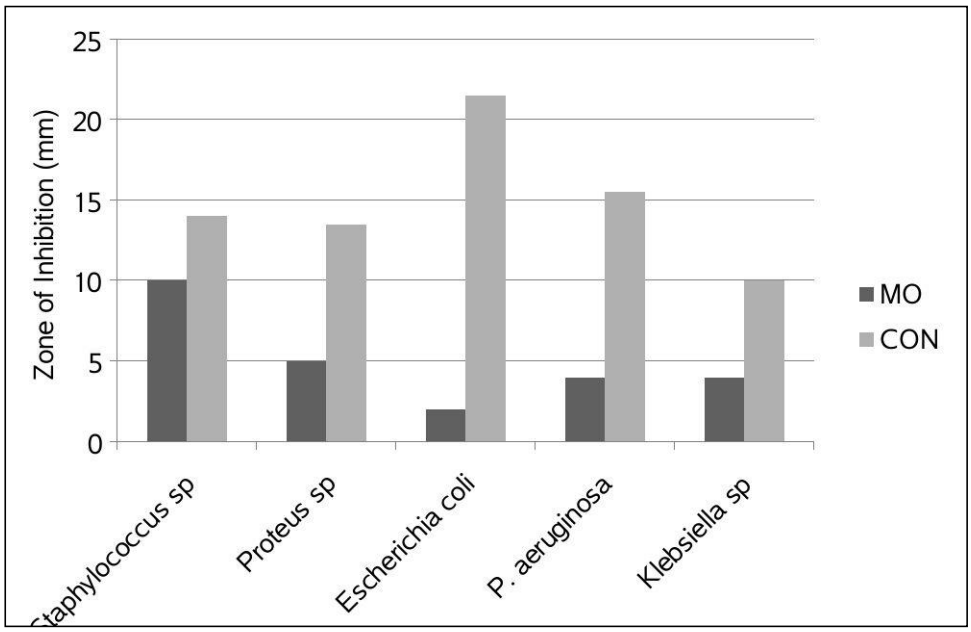
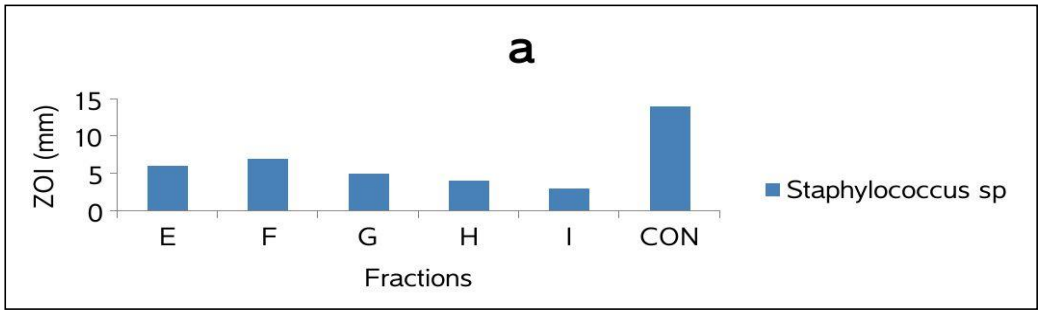
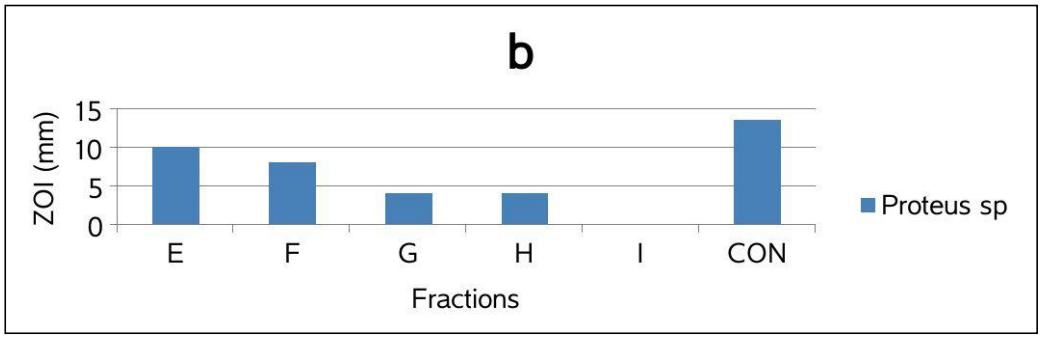


Figure 2b: Antibacterial potential of MO

Based on the foregoing results, crude extract, MO was subjected to column chromatography. Fractions 10-20 which were eluted with an increasing gradient of MeOH in EA (EA:MeOH, 1:0→0.3:1), were combined based on TLC evidence and combinations $E_{10\text{-}12}$, $F_{13\text{-}14}$, G_{15}, $H_{16\text{-}17}$, $I_{18\text{-}20}$ were obtained. Afterwards, 0.125g/ml of each fraction combination was prepared and examined for their antibacterial effect on the selected microorganisms, using agar well diffusion technique. Ciprofloxacin was used as the positive control. The antimicrobial activity of the chromatographic fractions are depicted in Figure 3.





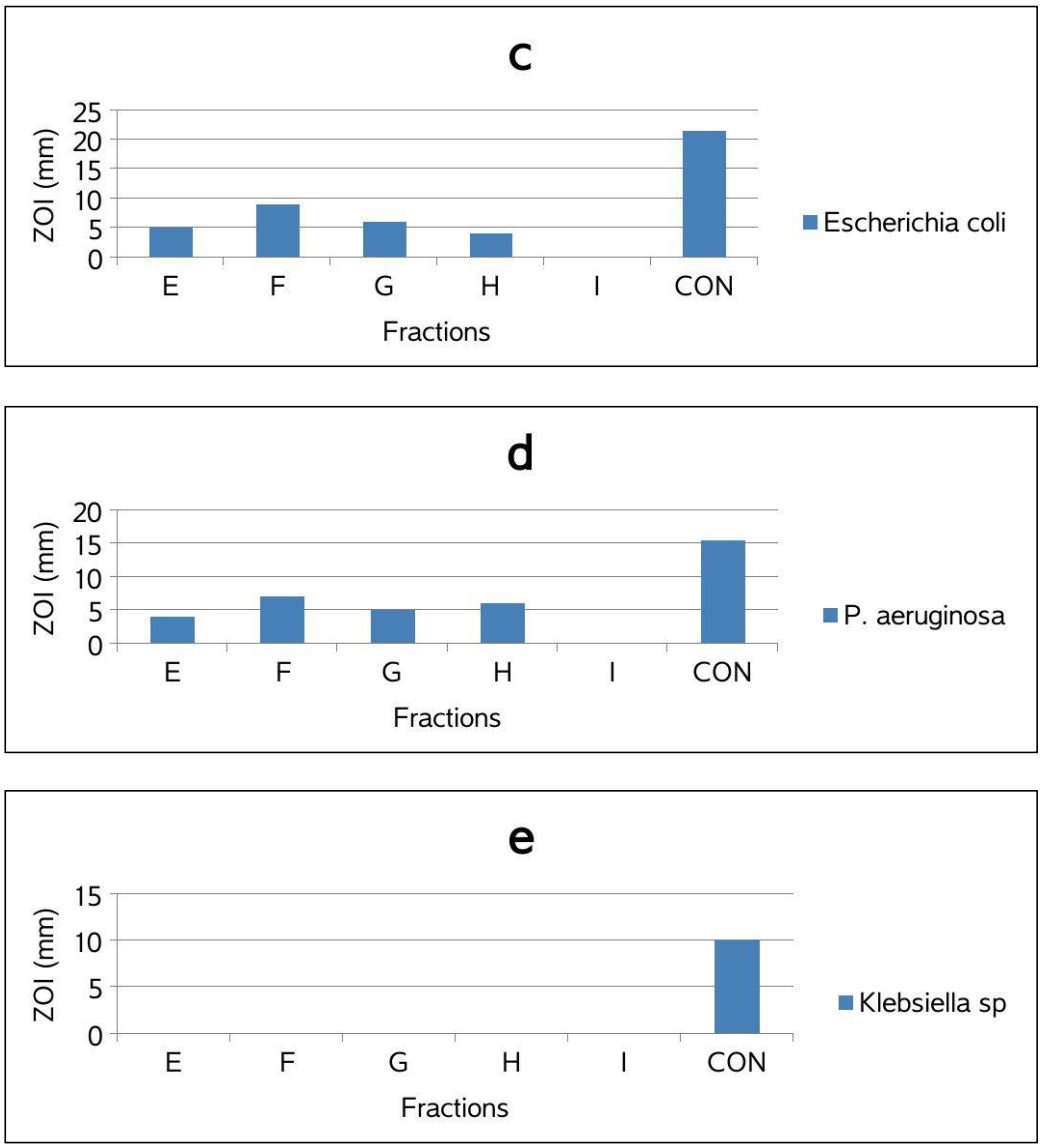


Fig. 3 a,b,c,d,e

ZOI= Zone of Inhibition
Figure 3: Antimicrobial potential of CC fractions from MO crude extract

From figure 3, it could be inferred that fractions E to I synergistically contributed the antibacterial inhibitory potential demonstrated by MO against *Staphylococcus* sp. because non of the fractions gave a ZOI greater than 10 mm. Similarly, the anti-*klebsiella* activity which was observed in figure 2b was lost after fractionation. In contrast however, column chromatographic fractions exhibited improved antimicrobial activity against the test organisms. Compared to extract MO, fractions E and F gave better activity against *Proteus sp*, Fractions E, F and G showed better activity against *E. coli* and fractions F, G and H were more active against *P. aeruginosa*. Out of all the fractions, fraction F seems to be the most active against the selected organisms. This could be as a result of the accumulation of saponins and some other antimicrobial phytochemicals in fraction F. The confirmation of the actual constitution of this active fraction is a subject of future spectroscopic investigation.
Although phytochemicals work in synergy against some specific bacteria, employing chromatographic fractionation of the crude extract could be a way of unmasking the biological activity of some useful phytochemicals.

4.0 Conclusion

The phytochemical screening of water melon rind extracts reveals the presence of secondary metabolites which have been earlier implicated in

antimicrobial therapy (Adelani-Akande *et al.*, 2015). This was especially ascertained by the moderate antimibicrobial activity exhibited by the methanolic extract (MO) and some of its fractions. Therefore, with further investigations, the usually discarded water melon rind could emerge as a rich source of new antimicrobial compounds with templates which are new to multi-drug resistant bacteria.

5.0 References

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