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ORIGINAL ARTICLE

Phytochemical analysis of watermelon (*Citrullus vulgaris*) and Detection of its Antimicrobial activity

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ABSTRACT
Despite the predominant view that watermelon is essentially water and sugar, watermelon is really a supplement dense food. It gives high amounts of vitamins, minerals, and antioxidants and it gives abnormal amounts of nutrients, minerals, and cell reinforcements and only few calories. The phytochemical analysis of watermelon(Citrullus vulgaris) extracts was carried out qualitatively using accepted laboratory techniques, in addition the antimicrobial activity of the plant extract against some selected bacteria (Bacillus subtilis, NCTC 8236, ACCC), Staphylococcus aureus (ATCC 25923 & CVCC), Escherichia coli, E. coli (ATCC 25922, ACCC), and one fungus (Candida albicans, ATCC 7596, CVCC) and fungi was investigated. The result indicated that the plant extract contain saponins, carotenoids, glycosides, alkaloids, sterols, triterpenes, phenolic compounds, carbohydrates and reducing sugars. The antimicrobial activity study showed strong susceptibility of watermelon (Citrus lanatus) against the tested microorganisms and it was also compared with selected antibiotics. However its activity varies with the nature of the solvent used for extraction, and the part of the watermelon plant and the tested microorganisms. The result of the present study indicated that plant of Citrulluslanatus contained bioactive components with potent antibacterial as well as antifungal activity.
***Keywords**: fatty acids ; watermelon ; antibiotics; chromatography/mass spectrometry (GC-MS) ; Automatic Amino Acid Analyser*

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INTRODUCTION

Fruits and vegetables are the most significant sources of phytochemicals. Phytochemicals use for both human diets and natural antimicrobial agents in food preservation. Their advantages for health are principally because of high antioxidant and antimicrobial activities. Around 200,000 phytochemicals are known up until now and 20,000 of them have been recognized as beginning from fruits, vegetables and grains [22].

Various epidemiological examinations have exhibited that high utilization of fruits and vegetables can give health advantages and lower rate of ailments like cancer and cardiovascular illness. It has been generally accepted for more than 20 years since this is because of their antioxidant constituents including carotenoids, flavonoids, phenolic compounds and vitamins. These phytochemicals were thought to "mop up" possibly unsafe free radicals when they were produced in excess in the body, because of disease or environmental stress [19].

It has been reported that all fruit juices including the watermelon juice show antimicrobial activities in dilutions extending from 1:2 to 1:16. Besides, it has been discovered that fruit extracts show higher antibacterial action than leaf extracts [19, 10].

At the point when the antimicrobial action of watermelon juice probioticated utilizing various strains of lactobacilli was compared against *Salmonella typhimurium*, it was found that all the lactobacilli could hinder growth *S. typhimurium* with *L. casei* being the most potent. *S. typhimurium* was completely destroyed in probioticated juice after 2-6h [10].

Watermelon juice may give a novel source of the essential amino acid, arginine. Arginine is a precursor for nitric oxide, which has been appeared to lower blood pressure, diminish blood clotting and protect against myocardial infarction and strokes [9]. Free amino acids, for example, phenylalanine, histidine, tryptophan, lysine, ornithine, arginine, aspartic acid, threonine, serine, glutamic acid, glutamine, citrulline, alanine, valine, isoleucine and leucine are reported to have been extracted from watermelon and quantitatively analyzed [25].

It has been reported that watermelon plant contains many phytochemicals [12, 7, 6, 20]. However, the antimicrobial compounds found in plants are of interest because antibiotic resistance is becoming a worldwide public health concern in terms of food borne illness and nosocomial infections Anderson *et. al.* [4]. The most important of these bioactive constituents of plants includes phenol, tannin, saponin, alkaloid, flavonoid, steroids, carotenoids, and cyanogenic glycosides [18]. These phytochemicals constitute the antibiotic principals of plants [3]. They are found to be distributed in plants [14]. The present study aimed to determine the phytochemical properties of watermelon plant and detection of its antimicrobial activity.

MATERIAL AND METHODS

All extraction solvents and chemicals used were of analytical reagent grade and were purchased from Beijing Chemicals, Beijing. All solvents for analytical analysis were of HPLC grade and were purchased from J & K Chemical Ltd, Beijing.

All parts of watermelon (*Citrullus vulgaris*), were harvested from Elselate-Eastern Khartoum Estate Research Centre, Sudan, and their identification was verified. Fruits were also purchased from local market Beijing and dried in an oven by standard-drying and air gravitation oven (DGG-9070A Shanghai Samsung Laboratory Instrument Co. Ltd.) at 48°C for 72 hrs, while other parts were air dried and ground to powder form and kept until use. In other methods, the parts were also freeze-dried and dried by nitrogen. The fruits and their fresh juices were also used.

Preparation of crude extract

Different solvents were employed for extraction. 100g of the powdered plant tissues were used. The roots, stems, leaves and fruits (all parts) were exhaustively extracted separately for 2 hours with petroleum ether, chloroform, ethyl alcohol, butanol, methanol and water in a soxhlet apparatus. Each sovent extract was filtered and evaporated under reduced pressure using Rota-vapor (Heidolph, Heizbad, Laborota 4001, Germany, 2000). The extracted plant material was then air-dried, repacked in the soxhlet apparatus and exhaustively extracted with methanol (80%) for 2 hours. The methanol extract was filtered and evaporated under reduced pressure using Rota-vapor.

The extracts were dissolved in dimethyl sulphoxide (DMSO) to make the final concentrations and were kept in a refrigerator for subsequent use. Simultaneously, water extract was prepared by adding 10ml of boiled distilled water to 5g of coarsely powdered plant leaves in a beaker and heated on a water bath with occasional stirring for 4 hours. The aqueous extract was then filtered and rewashed with small volume of boiled distilled water and added to the filtrate, which was then adjusted to 5ml volume and used immediately.

Phytochemical Analysis

Phytochemical analysis of the water melon extracts with various solvents extracts was accomplished qualitatively utilizing accepted laboratory techniques as described by Tiwari *et.al.* [26] and AOAC [5]. Phytochemicals tested included the presence of alkaloid, flavonoids, glycosides, saponins and tannins were conducted accordingly.

Preparation, isolation, and identification of antimicrobial organisms

Microbiological material

The standard microorganisms used for this study were received from the Medicinal and Aromatic Plants Research Institute, National Centre for Research, Sudan and from the Department of Microbiology, Beijing University of Chemical Technology, China. They were *Bacillus subtilis,* B.s. (NCTC 8236, ACCC), designated as Gram +ve, *Staphylococcus aureus,* S.a. (ATCC 25923 & CVCC), designated as Gram +ve, *Escherichia coli*, *E. coli* (ATCC 25922, ACCC), designated as Gram -ve, and one fungus, *Candida albicans*, Ca.a. (ATCC 7596, CVCC), designated as Gram +ve.

Preparation of the test organisms

Preparation of standard bacterial and fungal suspensions

The average number of viable organisms per ml of the stock suspensions was determined by means of the surface viable counting technique. 10^6 colony-forming units per ml were used. Each time, a fresh stock

suspension was prepared; the experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.
The fungal culture *Ca.a* was maintained on Saboraud dextrose agar, incubated at 25ºC for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspended in 100mL of sterile normal saline, and the suspension was stored in a refrigerator for subsequent use.

In vitro antimicrobial activity

The cup-plate agar diffusion method was adopted to assess the antibacterial activity of the prepared extracts. 0.6 ml of standardized bacterial stock suspensions (10^6) colony-forming units per ml (cfu/ml) was thoroughly mixed with 60 ml of sterile nutrient agar. 20 ml of the inoculated nutrient agar were distributed into sterile Petri dishes. The agar was left to set and in each of these plates, 4 cups, 10 mm in diameter, were cut using a sterile cork borer No. 4, and the agar discs were removed.
Alternate cups were filled with 0.1ml of each extracts using microtiter-pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37ºC for 18 hours. Two replicates were carried out for each extract against each of the test organism. Simultaneously addition of the respective solvents instead of extracts was carried out as controls. After incubation, the diameters of the zones of inhibition were measured to the nearest mm, averaged and the mean values were tabulated.

RESULTS

General phytochemical screening of all parts of the watermelon plant (SP1)

The results for the general phytochemical screening of all parts of the watermelon plant designated as SP1 are shown in Table (1) below. Phytochemical screening of the aqueous and other organic solvents extracts show that,S terols and triterpenes**,** Carotenoids**,** Reducing compounds, Saponinns, glycosides, Alkaloids compounds and Carbohydrates were present in the extracts with varying amounts. In the present study, results showed that various parts of watermelon plant contain different phytochemical compounds such as Sterols triterpenes, Glycosides and Saponinns.

Antimicrobial activity of the watermelon plant

The antimicrobial activities of the water and solvents extracts of all parts of the watermelon plant are shown in Table (2) and Fig. (1 and 2). The highest antimicrobial activity was only against the *E. coli* bacteria. Their respective inhibition zones had diameters of 20 mm and 22 mm. However, the methanol extracts of all parts of the watermelon plants showed very low antimicrobial activity against the fungus *Candida albicus*. On the other hand, the antimicrobial activities of all parts of the watermelon plant in methanol extracts are shown white fruit crust and the seeds were highest antimicrobial activity against the *E. coli*. Their respective inhibition zones had diameters of 20 mm and 22 mm. However, the methanol extracts of all parts of the watermelon plant showed very low antimicrobial activity against the fungus, *Candida albicus*.

Table 1 General phytochemical screening of all parts of SP_1

SN	Class of chemical compounds	SP_{1a}	SP_{1b}	SP_{1c}	SP_{1d}	SP_{1e}	SP_{1f}	SP_{1h}
1	Sterols and triterpenes	+	+	+	+	+ +		+++
2	Carotenoids	-	+	+ +	+ +	+	+++	+
3	Reducing compounds	+ +	-	+ +	+	+ +	+++	++
4	Saponinns (saponosides)	+	±	+ +	-	+	-	-
5	Glycosides	+	-	++	+++	++	++	+++
6	Alkaloids compounds	+	±	++	+ +	+ +	+	
7	Phenoloic compounds	+	±	++	+ +	+	+	
8	Carbohydrates	+	+ +	+	-	-	-	+

Watermelon plant = (SP1); SP_{1a}= Roots; SP_{1b}=Stem; SP_{1c}= Leaves; SP_{1d} = Green crust; SP_{1e} = White crust; SP_{1f}= kernel; SP_{1h} = seed

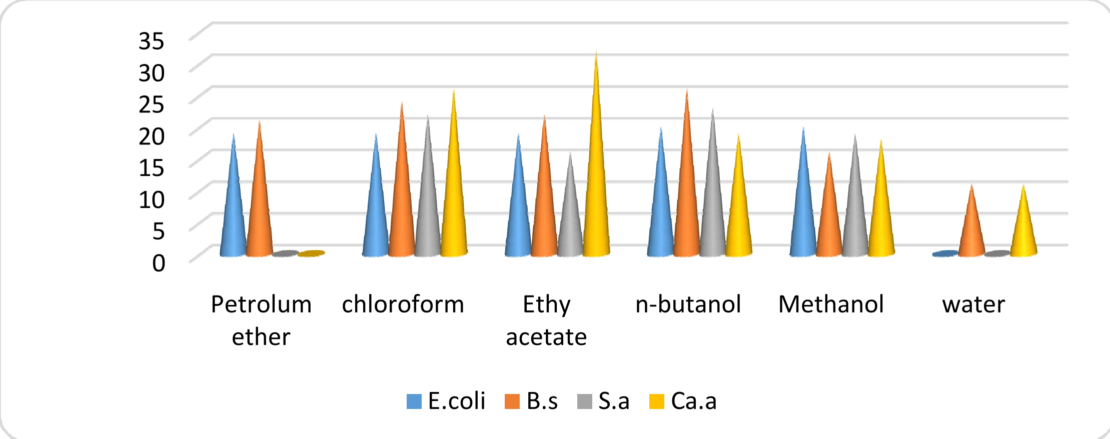

Fig. 1. Antimicrobial activity of all parts of the watermelon plant in different solvents

A B C

Fig. 2. Growth inhibition of *Staphylococcus aureus*(A), *Bacillus subtilis* (B) bacteria and *Candida albicans* yeast against water melon plant

Table 2 Antimicrobial activity of CH_3OH all Watermelon plant parts

Watermelon plant parts	*E.coli*	*Bacillus subtilis*	*Staphylococcus aureus*	*Candida albican*
SP_{1a}	0	17	0	0
SP_{1b}	0	15	15	11
SP_{1c}	0	16	17	13
SP_{1d}	19	17	19	15
SP_{1e}	20	15	15	0
SP_{1f}	18	16	0	11
SP_{1h}	22	18	15	11

Watermelon plant = (SP1); SP_{1a}= Roots; SP_{1b}=Stem; SP_{1c}= Leaves; SP_{1d} = Green crust; SP_{1e} = White crust; SP_{1f}= kernel; SP_{1h} = seed

As shown in Fig. (2), the Petroum ether exhibited more potency on *Bacillus subtilis* and *E. coli* bacterial in contrast to the aqueous water extract which produced less effect on *Bacillus subtilis* and *Candida albicans.* On the other hand, other solvents extracts (chloroform, ethanol, butanol and methanol) exhibited high potency on *E. coli*, *Bacillus subtilis(B.s*), *staphylococcus aureus* (S.a) bacteria and *Candida albicans fungus (Ca. a*). In addition, *Ca. a* was the most inhibited microorganism compared with the other tested microorganism. Moreover, the ethanol extract was the most efficient solvent. When comparing with the findings of other researchers, Braide, *et al*. [7] observed that aqueous extracts shows better response to the antibacterial activities than the ethanol whereas Nwankwo *et. al.* [20] reported the contrary. This inconsistency may be a component of methodological contrasts and strain inconstancy.

DISCUSSION

Seeming well and good for looking through the new wide range antimicrobial to treat the antimicrobial illness much consideration has been engaged toward plant extracts and biologically active compounds

confined from famous plant species. The utilization of medicinal plants plays a unique role in covering the essential health needs in developing countries and these plants may offer a new source of antibacterial, antifungal and antiviral agents with noteworthy activity against infective microorganism [23].
Plants are a noteworthy source of medication from which most medication mixes exude. Therapeutic phytochemicals have utilized as model possibility for the blend of concoction medications and pharmaceuticals. Late increment in logical knowledge of the chemical composition and action of plant mixes brought about potential chances to fix a wide assortment of diseases [16].
The role of these phytochemicals as antimicrobial has been reported by many researchers [15, 21, 24, 6]. Presence of phytochemical in watermelon plant was reported by many authors including [6, 7, 12, 20]. However, the variation in the types and amounts of phytochemicals and other active ingredients could be affected by the geographical location. Existence of these phytochemicals in the extract was an unmistakable sign of antimicrobial possibilities of the various parts of watermelon plant. Antibacterial activity of the plant extracts demonstrated that all tested microorganisms were susceptible.
The present study, demonstrated antimicrobial activity of petroleum ether, chloroform, ethanol, ethyl alcohol, butanol methanol of roots, stems, leaves, green crus, white crust, kerneland seeds from watermelon against bacteria (*Escherichia coli*, *Staphylococcus aureus* and Bacillus subtilis and fungi (*Candida albican*). These results were in agreement with those reported by Adelani *et. al*., [1], Gupta Alka *et. al*, [13].
Almost all extracts of watermelon plant including water extract except ether extract showed significant antifungal activity. Earlier study on the primary phytochemical screening of these extracts demonstrated the presence of wide range of phytoconstituents along with antifungal compounds.
Susceptibility of *Staphylococcus aureus* and *E. coli* showed in the present study agreed with that Joana Monte *et. al*., [17] who demonstrated the potential of phytochemicals to control the growth of ***these microorganisms*** in both planktonic and biofilm states. Adunola *et al.* [2] attributed susceptibility of *S. aureus* to presence of saponins in watermelon plant. Therefore, our findings therefore, support the view that other phytochemicals are active against Gram positive bacteria also.
Several researchers examined the impact of plants extracts and their active ingredients as antimicrobial agents to control harmful bacterial development. Some researchers proposed that antimicrobial compounds of the plant extracts such as terpenoid, alkaloid and phenolic compounds, interfaces with proteins and enzymes of the cell membrane making its disruption to disperse a flux of protons towards cell exterior which promptscell death or may restrain enzymes vital for amino acid biosynthesis [8, 11].

CONCLUSION

The present investigation legitimizes that extracts of all parts of watermelon plant have promising activity against a wide scope of microorganisms in charge of most common microbial diseases and infections and thus give prospect of finding new clinically powerful antimicrobial mixes. In this manner, further research is important to recognize the capable mixes inside these plants and to decide their full range of adequacy too. Moreover, as plants contain an assortment of phytochemicals, proficient extraction, isolation, and purification procedures are required to get the ideal antimicrobial phytochemical so as to explicitly describe its potential adequacy. Isolation and purification of phytochemicals in the extract may likely exert more effect like the commercial antibiotic.

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