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GC-MS Analysis of Phytoactive Compounds, Antioxidant and Antibacterial Activity of *Citrullus lanatus* Seeds

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The current study investigated the therapeutic potential of Citrullus lanatus seeds which are commonly discarded after eating the fruit. In this day and age, plant secondary metabolites are preferred therapeutic agents to manage a variety of diseases and disorders. The present study aimed to investigate the bioactive secondary metabolite profile of Citrullus lanatus seeds by investigating total phenolic and flavonoid content, antioxidant potential, and Gas Chromatography-Mass Spectrometry (GC-MS) analysis of bioactive compounds and antibacterial properties of four different crude extracts. Alkaloids, flavonoids, phenols, steroids, tannins, saponins, phytosterols, terpenoids, and glycosides were revealed in the seeds after qualitative phytochemical examination utilizing several solvents of varying polarity and established techniques of analysis. DPPH radical scavenging assay was used to assess the antioxidant potential and the total flavonoid and phenolic contents in seed extracts were determined using the spectrophotometric method. Methanolic extract revealed higher extractive yield, antioxidant potential, a higher total phenolic content (132.68 ± 0.861 mg of GAg), and higher total flavonoid content (48.13 ± 0.451 mg of Qg) as compared to other extracts. Gas Chromatography-Mass Spectrometry (GC-MS) analysis of all four seed extracts revealed the presence of 27 high and low molecular weight chemical entities in toto with varying amounts. These bioactive chemical substances have been revealed to be physiologically significant and essential from a pharmaceutical standpoint. This research demonstrates that the Citrullus lanatus seeds are high in bioactive secondary metabolites that are beneficial to human health, have a high antioxidant capacity, and antibacterial action against certain bacterial strains, indicating that these seeds have a lot of therapeutic value.

Keywords: Antioxidants; Antibacterial activity; Citrullus lanatus; 1, 1-diphenyl-2-picrylhydrazyl (DPPH); GC-MS; Secondary metabolites.

The major objective of introducing plant-based bioactive compounds into healthcare is to create a healthy interaction with the body's chemistry while avoiding the unwanted and off-target side effects that medications are known for[1]. Pharmacognostical researchers are being forced to seek out novel plant-based bioactive compounds for use in the treatment of a wide range of diseases and disorders due to an increase in the human population, insufficient drug supply, unmanageable treatment costs, and an increase in antimicrobial resistance to currently used drugs. Plants provide the majority of foods, pharmaceuticals, and nutritional supplements[1, 2]. Primary and secondary

metabolites are the chemical compounds found in plants and are termed phytochemicals. Because they are involved in activities like cell division, proliferation, reproduction, metabolism, storage, and development, primary metabolites are vital for plant life. Secondary metabolites in plants aren't merely waste byproducts of primary metabolism; they also have a big influence on plant defenses, ecology, and evolution[2]. Flavonoids are predominant and paramount secondary metabolites found in plants. Flavonoid is a generic name used to describe a family of over 6000 compounds that have a 15-carbon skeleton with two phenyl rings A and B and a heterocyclic ring C. The oxygen with heterocyclic ring C mediates the joining of rings A and B. C6-C3-C6 is the chemical structure used to denote such a compound. Because of their structure, they are essential variable phenolic compounds with strong anti-oxidant properties. Plants resist oxidative damage because of their inherent secondary metabolites, and as a result, when ingested, they constitute a dietary supply of anti-oxidants[3]. They might minimize the amount of ROS in stressed systems by acting as effective singlet oxygen quenchers. Secondary metabolites have received a lot of attention in pharmacognosy research in the last two decades because they've been shown to have anti-inflammatory, anti-carcinogenic, anti-tumor, anti-oxidative, anti-hypertensive, anti-viral, anti-aging, cardioprotective, and immunomodulatory properties, as well as the ability to modulate enzymatic functions, inhibit cell proliferation, induce apoptosis, and inhibit bacterial and fungal growth among others. Current research is also highlighting their role as potent along with their potential to act as substrates for biochemical reactions, cofactors for enzymatic reactions, ligands that antagonize or agonize cellular receptors, act as prebiotics, act as immunomodulators, etc. Bioinformatics and molecular docking information are being applied to forecast the possible use of secondary metabolites in human health and illness[4].

Plant secondary metabolites (PSM) are naturally occurring physiologically active substances utilized in the traditional type of medicine and diversification of industrial uses. In the case of chronic illnesses, prevention is a better method than therapy. Plant-based foods, such as fruits, have high levels of bioactive phytochemicals, which may have health advantages beyond basic nutrition, such as lowering the risk of chronic illnesses[5]. Watermelon (*Citrullus lanatus*), a *Cucurbitaceae* family fruit with over 1,000 cultivars, is a widely farmed fruit worldwide. The large edible fruit, which is a berry with a tough rind and no internal sections and is botanically referred to as a pepo, is grown at optimum conditions all over the world, from subtropical to temperate regions. Although seedless cultivars exist, the luscious, juicy flesh is generally deep crimson to pink, with abundant black seeds. The rind is edible after cooking, and the fruit can be eaten fresh or pickled. It can also be drunk as a juice or as part of a mixed drink[6]. This fruit is a good choice for a healthier diet since it contains phytochemicals such as terpenoids, glycosides, alkaloids, flavonoids, flavonoids, coumarins, quinones, carotenoids, lycopene, anthocyanins, and phenols, as well as vitamins and minerals[7]. Regular consumption of this fruit, which is high in health-promoting substances, may reduce the risk of a variety of deadly diseases, including diabetes, cardiovascular disease, liver problems, obesity, cancer, neurological disorders, and aging-related conditions[8]. Considering the beneficial effects of plant bioactive compounds on human health, this study was organized to determine the presence of secondary metabolites (phenolic and polyphenolic compounds), free radical scavenging activity, and antibacterial activity in *Citrullus lanatus* seeds that are typically discarded after fruit consumption. These seeds are high in bioactive phenolic and polyphenolic components such as flavonoids, phenols, saponins, terpenoids, glycosides, steroids, alkaloids, coumarins, and quinones, according to the findings of this study. All of the extracts of the seeds were shown to have substantial antioxidant properties as well as antibacterial activity against some of the microbial strains.

MATERIALS AND METHODS

Collection of *citrullus lanatus* seeds

In May, *Citrullus lanatus* fruits were obtained at a local market in Dharmapuri Mandi, Dehradun, and their seeds were harvested. Only healthy-looking fruits were selected. Before analysis, seeds were shade dried and kept in a conducive environment.

Chemicals

The reagents used for the study were acetone, methanol, chloroform, Mayer's Reagent, Ammonia Solution, ferric chloride, concentrated H_2SO_4, quercetin, ascorbic acid, gallic acid, lead acetate, 2, 2-diphenyl-1-picryl-hydrazyl (DPPH), sodium carbonate, Folin-Ciocalteau's phenol reagent, aluminium chloride, potassium acetate, hydrogen peroxide, Mueller-hinton agar (MHA), nutrient broth, peptone water, procured from Himedia, Merck, and sigma.

Preparation of plant extract

The removal of phytochemicals requires extraction. The solvents utilized and the chemical properties of samples are the two most critical elements that determine the extraction yield under the same time and temperature circumstances. Several studies have shown that extractive yield varies depending on the solvent used[9]. Seeds were collected and cleaned thoroughly with tap water before being sterilized with distilled water. After that, the seeds were shade dried at room temperature for 7-8 days, then homogenized into a fine coarse powder with an electric blender, and lastly kept in airtight containers until needed. Methanol [M], Chloroform [C], Acetone [A], and water [AQ] were the various solvent systems of varying polarity used to extract the fine seed powder using the hot maceration technique. In the conical flasks, 20 grams of dry powder were poured in 100 mL of each solvent, plugged with cotton wool, and shaken at 120 rpm for 38 hours on a rotary shaker. After 38 hours, the extract was filtered with sterilized Whatman Filter Paper Grade No 1. While the solvent was being evaporated, the supernatant was being collected. The resulting blackish gummy exudates residues were weighed using a balance to calculate the extractive yield. The crude extract was maintained at 4°C in sealed Eppendorf tubes before being subjected to qualitative phytochemical analysis, total phenol and flavonoid content, and antioxidant and antibacterial properties testing[10].

Phytochemical preliminary screening

Traditional procedures established by Trease and Evans in 2002[11] were used to test the extracts for phytochemicals (secondary). The qualitative assessments were carried out to confirm the presence or absence of Flavonoids, phenols, alkaloids, steroids, saponins, glycosides, phytosterols, terpenoids, triterpenoids, anthraquinones, and tannins. The results of all the above tests are summed up in results section.

Assessment of total phenol content (TPC)

The method of assessing the quantity of phenolic content in samples is known as TPC activity. The redox characteristics of phenolic chemicals found in plants allow them to serve as antioxidants[6, 10, 11]. Total phenolic content was estimated via Folin-Ciocalteau's reagent assay as reported by McDonald et al[12]. 0.2 gram of extracts was dissolved in 1 ml of their respective solvents. 1 ml of this solution and 0.1 ml (0.5 N) Folin-Ciocalteau's reagent was combined and the reaction mixture was incubated at room temperature for about 15-20 min. After that 3 ml, saturated sodium carbonate solution was poured and again incubated for about 30 min. at room temperature. Finally, the absorbance was taken at 760 nm. Gallic acid was employed as a positive control for which a standard curve was developed beforehand.

Assessment of total flavonoid content (TFC)

The flavonoid content was measured using the colorimetric technique with aluminium chloride[13]. The reaction mixture was incubated at room temperature for 30 minutes, with 1.0 ml of sample (1 mg/ml), 1.0 ml methanol, 0.5 ml of (1.2 percent) aluminium chloride, and 0.5 ml of (120 mM) potassium acetate in a final volume of 3 ml. At 415 nm, the absorbance of all the samples was measured. As a positive control, quercetin was utilized.

Antioxidant assay

DPPH free radical scavenging activity

The free radical scavenging activity of *Citrullus lanatus* seed extracts was determined by using the 2, 2- Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method[10, 14]. DPPH in oxidized form gives a deep violet color in methanol. An antioxidant compound donates the electron to DPPH thus causing its reduction and in reduced from its color changes from deep violet to yellow[15]. All extracts were measured for hydrogen donating or radical scavenging ability. Extracts were diluted to obtain concentrations of 0.1, 0.3, 0.6, 0.8 and 1.0 mg/ml. Diluted extract solutions (1 ml each) were assorted with an ethanolic solution of DPPH (0.004%). After 30 min of incubation at room temperature, the reduction of the DPPH free radical was spanned by reading the absorbance at 517nm using UV-Visible Spectrophotometer.

Initially, absorption of a blank sample containing an equal amount of ethanol and DPPH solution was prepared and measured as a control. Ascorbic acid was used as standard. The experiment was carried out in triplicate. Percentage inhibition was calculated using equation (1). The data were presented as mean values ± standard deviation (n = 3).

$$\% \text{ inhibition} = [\text{ Absorbance of control - absorbance of sample / Absorbance of control }] \times 100 \quad ...(1)$$

The GC–MS Analysis

Agilent Technologies GC system with GC-7890A/MS-5975C model (Agilent Technologies) integrated with HP-5MS column (30 m in length, 250 mm in diameter, 0.25 mm in film thickness) was used to analyze bioactive compounds from various *Citrullus lanatus* seeds extracts. An electron ionization device using high-energy electrons (70 eV) was used for spectroscopic evaluation by GC–MS. The gas phase was pure helium gas (99.995% purity) at a flow rate of 1 mL/min. The starting temperature was maintained at 50–1500 with a 30 C/min increase rate and a 10-minute hold duration. Subsequently, the temperature was raised to 300°C at a rate of 100°C per minute. After syringe filtration in splitless mode, one microliter of the obtained extracts diluted with appropriate solvents was loaded into the system. Based on the obtained peak area in the chromatogram, the relative quantity of bioactive compounds contained in each of the extracts was represented as a percentage. Based on GC retention time on HP-5MS column and spectral comparison with computer software data of standards (Replib and Mainlab data of GC–MS systems), bioactive chemicals extracted from various extracts of *Citrullus lanatus* seeds extracts were recognized.

Antibacterial assay

The antibacterial potential of *Citrullus lanatus* seeds extracts in different solvents was tested against Gram-positive bacteria *Bacillus cereus* 10451 (BC), *S. aureus* ATCC29213 (SA), and Gram-negative bacteria *Escherichia coli GIM1.708* (EC), *Escherichia coli DH5-Alpha* (ECá), *Salmonella enteritidis*10982 (SE). Agar well diffusion assay was incorporated to assess the antibacterial potential of all extracts of *Citrullus lanatus* seeds[16]. One ml of each bacterial culture was pipetted out and aseptically swab cultured on Mular -Hinton agar plates. Antibiotics chloramphenicol and tetracycline were used as a positive control. DMSO at a concentration of 15% was used as a negative control. All the extracts (0.2 ml) were loaded in the wells punched in the Agar. The MHA plates were incubated for 24 hours at 37^0 C and thereof looked for the zones of inhibition.

Statistical data

All the experiments were performed at least three times. The results were presented as mean ± S.E.M. (standard error of the mean).

RESULTS

Extractive yield result

The yield of crude extracts was determined in percentage using the following formula[9, 17].

$$\text{Yield (\%)} = (\text{Dry weight of extract} \div \text{Dry weight of plant material}) \times 100$$

The extractive yield of samples in four different solvents viz., methanol, chloroform, acetone, and water are given in Table 1.

The extractive yield varied among the different solvent systems incorporated. Among all the extracts, methanol extract showed the highest extractive yield as compared to other extracts.

Table 1. Extractive yield of *Citrullus lanatus* seed extracts in different solvents

Seed Extract	Yield w/w (%) Methanol[M]	Chloroform [C]	Acetone [A]	Distilled water [DW]
	10.42	2.94	5.10	7.96

Qualitative phytochemical screening result

All the seed extracts were undertaken for qualitative phytochemical analysis using different tests previously described. The results of the phytochemical analysis showed the presence of flavonoids, steroids, alkaloids, phenols, terpenoids, quinones, tannins, glycosides, and saponins in high amounts, while anthraquinones were not detected (Table 2).

Total phenolic content result

As a basis, phenolic content was measured using the Folin–Ciocalteu's reagent in each extract. The results were derived from a calibration curve ($y = 0.0079x - 0.2866$, $R^2 = 0.9861$) of gallic acid (50–250 µg/mL) (Fig.1) and expressed in gallic acid equivalents (GAE) per gram dry extract weight. The total phenolic contents of all the samples ranged from 54.87 to 132.68 mg/g gallic acid equivalent (Table 3). The content of phenolic compounds was higher in methanol extract and lower in chloroform extract

Total flavonoid content result

The concentration of flavonoids in all the four *Citrullus lanatus* seeds extracts was analysed via the spectrophotometric method with aluminium chloride. The concentration of flavonoids was expressed in terms of Quercetin equivalents (mg of Q/ g of extracted compound) for which the standard

Table 2. Phytochemical screening of *Citrullus lanatus* seeds extracts in different solvents

S. No.	Phytochemical constituents	Aqueous Extract	Acetone Extract	Chloroform Extract	Methanol Extract
1	Flavonoid	+	++	-	++
2	Phenols	++	++	++	++
3	Alkaloids	+	-	-	+
4	Steroids	++	+	-	+
5	Saponins	-	+	-	-
6	Glycosides	++	-	+	++
7	phytosterols	-	-	-	-
8	Terpenoid	++	+	+	++
9	Triterpenoid	+	-	+	++
10	Anthraquinone	-	-	-	-
11	Tannins	-	-	-	+

+ = present, + + = relatively abundant, - = not detected

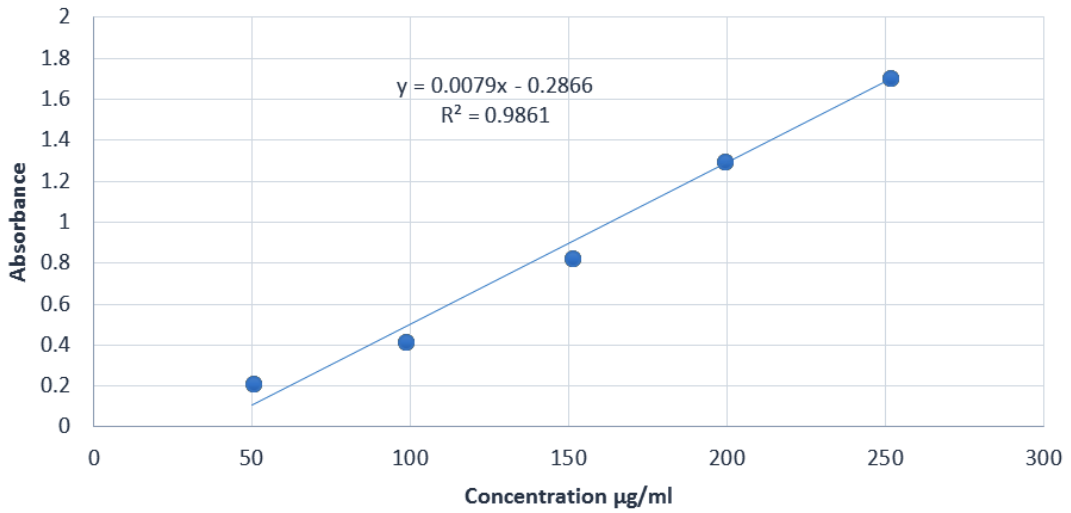

Fig. 1. Standard curve of Gallic acid (765 nm)

curve equation was (y = 0.002x - 0.0076, R^2 = 0.9988) (Fig.2). The concentration of flavonoids in all samples ranged from 13.65 to 48.13mg/g quercetin equivalent (Table 4). The flavonoid content was higher in methanol extracts and lower in acetone & chloroform extracts.

Antioxidant activity result

The DPPH radical scavenging activity of Methanolic [M] extract, Chloroform [C] extract, Acetone [A], and Aqueous [AQ] extracts of *Citrullus lanatus* seeds was detected and juxtaposed with Ascorbic acid as standard (Fig.3). The percentage inhibition (% inhibition) at various concentrations (0.1- 1.0 mg/ml) of all 4 samples as well as standard Ascorbic acid (40 to 200 ìg/ml) were calculated and plotted in graphs using Microsoft Office Excel 2018. Our result revealed that % inhibition of methanol extract is higher among all when compared to standard L-ascorbic acid (Fig. 4 & 5).

All the extracts in different solvents showed significant antioxidant potential when compared to the reference antioxidant ascorbic acid in a dose-dependent manner. IC50 value, representing the amount of extract which scavenged/reduced 50% of the DPPH radical, was calculated from the percent scavenging versus concentration curve. A higher concentration to reduce 50% of DPPH solution showed lower antioxidant activity. In this assay, the IC50 value of the reference standard ascorbic acid was found to be 114.62µg/ml while the IC50 value of different values varied. (Fig. 4)

Bioactive compounds contained in the extracts

Tables 5-8 list the bioactive chemicals found in methanol, water, chloroform, and acetone extracts of *Citrullus lanatus* seeds. Their elution sequence in an HP-5MS column was used to identify and characterize them. These bioactive chemicals' elution time, molecular formula, and quantity also are reported. Figures 6–9 show the GC chromatograms of the four extracts, which indicate the retention time in the column as well as the observed peaks that correlate to the bioactive chemicals contained in the extracts. Watermelon seeds when extracted with methanol

Table 3. Total phenolic contents in the extracts expressed in terms of gallic acid equivalent (mg of GA/g of extract)

Citrullus lanatus seed extracts	Total phenolic content mg of GA/g of extract
Water	94.78 ± 0.396
Acetone	65.98 ± 0.533
Chloroform	54.87 ±0.673
Methanol	132.68 ± 0.861

Each value is the average of three measurements ± (standard deviation)

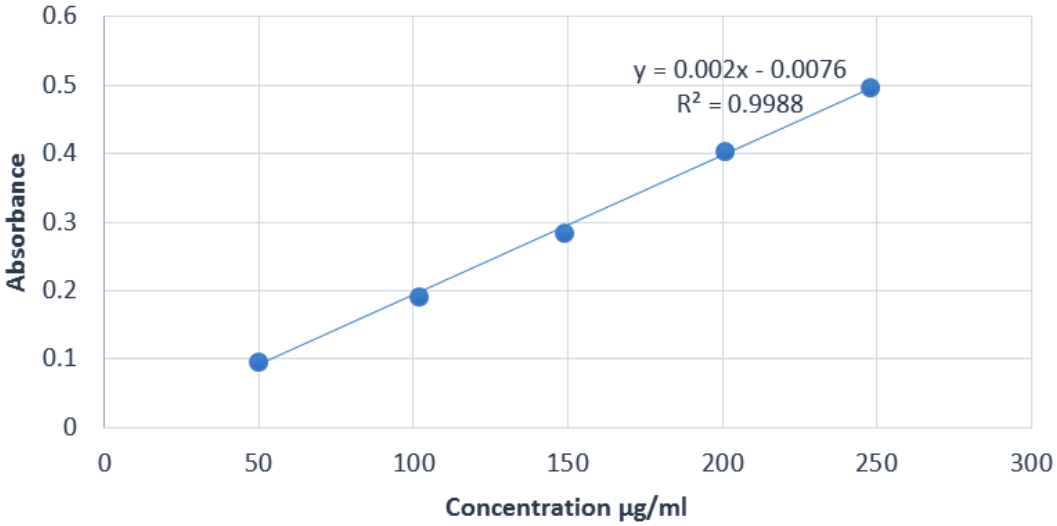

Fig. 2. Standard curve of Quercetin (510nm)

were found to be rich in Cis-7-Dodecen-1-yl acetate (17.29%), n- Hexadecanoic Acid (15.78%), beta - Sitosterol (14.16%), and Lupeol (8.48%). Other compounds that correspond to other peaks in the methanol extract chromatogram are chatted in (Table 5). Methyl tetradecanoate (32.76%), 9 octadecenoic acid(9.95%), and 4H-Pyran-4-one,2,3-dihydro-3,5- dihydroxy-6-methyl- (8.20%) were major abundant compounds in aqueous extract. Cyclohexene,1-pentyl-4-(4-propylcyclohexyl)- (36.87%) followed by 2- Chloroethyl linoleate (33.64%) were the major compounds in chloroform extract. 1-Azabicyclo(3,1,0) hexane (34.09%) and 1-Cyclohexyl-1-propyne(17.86%) were identified as major bioactive compounds in acetone extract.

Antibacterial activity result

Antimicrobial activity was assessed by the development and measurement of the inhibition zone around the discs after the incubation time. The interpretation of the results is shown in Table 9.

Table 4. Total Flavonoid contents in the extracts expressed in terms of Quercetin equivalent (mg of Q/g of extract)

Seed extracts	Total Flavonoid content mg of Q/g of extract
Water	41.36 ± 0.396
Acetone	16.88 ± 0.531
Chloroform	13.65 ± 0.820
Methanol	48.13 ± 0.451

Each value is the average of three measurements ± (standard deviation)

DISCUSSION

In May 2021, *Citrullus lanatus* seeds were gathered and analyzed. The presence or absence of secondary metabolites, antioxidant, and antibacterial properties of *Citrullus lanatus* seeds were investigated in this study. Phytochemicals exhibit biological features such as antioxidant activity, antibacterial activity, detoxification enzyme modulation, immune system modulation, and general hormonal activity regulation[3, 9, 13]. The extraction of phytochemical content is affected by the kind of solvent used and the procedure used to prepare the extract. Methanolic extract resulted in the highest extraction yield and a more complex phenolic content[17, 18]. Methanolic and water extracts showed the greatest extraction yield, high secondary metabolite extraction, high flavonoid content, high antioxidant potential, and effective antibacterial activity, according to the findings of this study. Terpenoids, glycosides, steroids, alkaloids, flavonoids, coumarins, and quinones were found in high concentrations in this research, however, phytosterols and anthraquinones were not. This is consistent with Ali et al., 2012[19], who found that alkaloids and terpenes are extensively dispersed throughout the Citrullus genus. For methanol extract of *Citrullus lanatus* seeds,

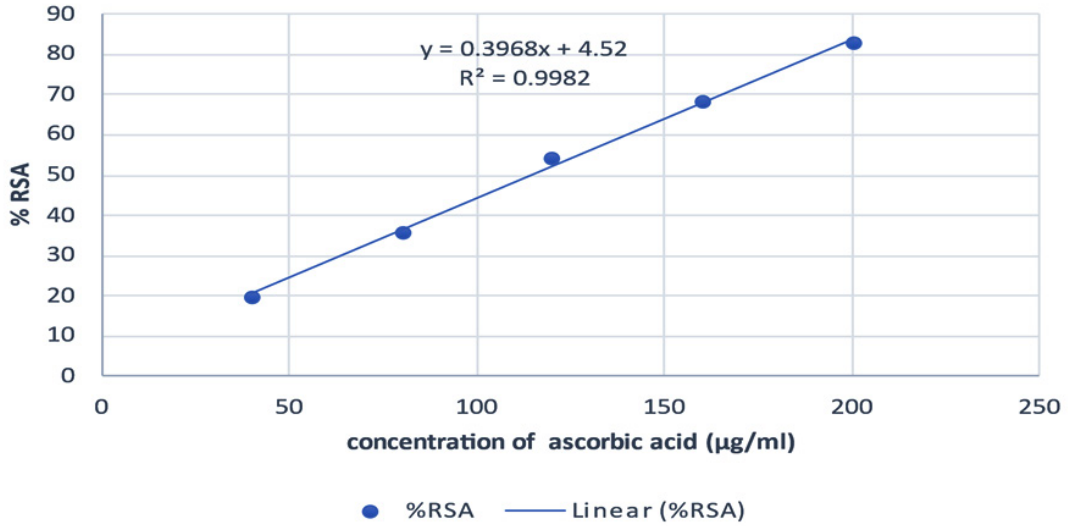

Fig. 3. %Inhibition of standard (L-ascorbic acid 40 to 200 µg/ml)

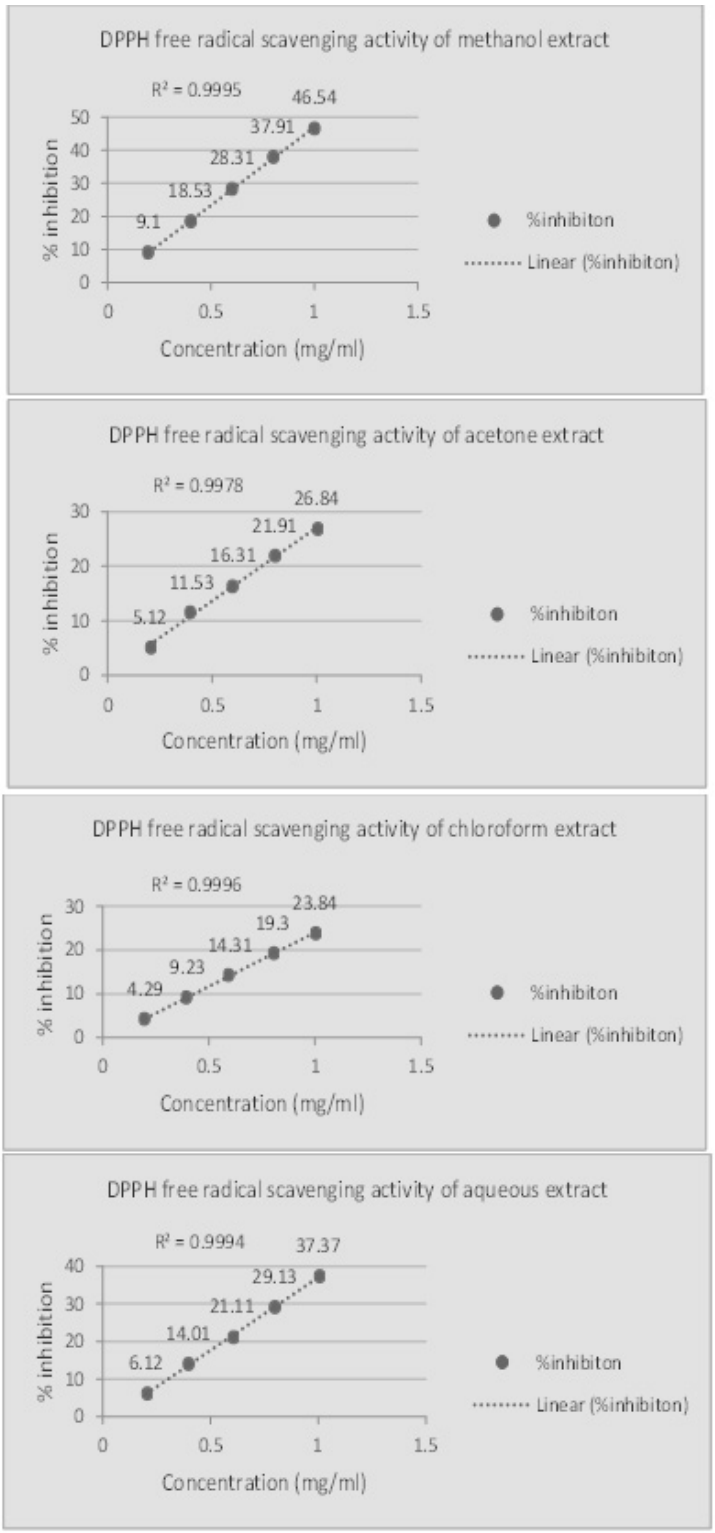

Fig. 4. Graphical representation of comparative percent inhibition of DPPH free radical by *Citrullus lanatus* seed extracts in four different solvents

Omoboyowa et al. 2015[20] reported flavonoids (2.310mg/100g), phenol (1.371mg/100g), saponins (1.553mg/100g), alkaloid (33.795mg/100g), and tannins (0.536mg/100g). Gwana, *et al*. 2014[21] also found flavonoids 0.01 percent, phenol (GAE) 0.01 percent, saponins 0.09 percent, alkaloid 0.91 percent, and tannins 0.04 percent in the phytochemical percentage composition of the Rosmas type of watermelon seeds. The bioactive chemicals found in the sample have been shown to have pharmacological and physiological properties. These seeds contain significant free radical scavenging activity and consequently antioxidant activity, according to our research. The amount and kind of bioactive compound produced by medicinal plants determine its anti-ailment action and different physiological impacts on the human body system. The range of this work, however, did not include an exploration into the precise functions of the extracted phytochemicals from watermelon seed; studies have revealed that these secondary metabolites are subject to a range of pharmacological effects in fruits and vegetables[22,23]. Because of the companionship of alkaloids, watermelon seeds can be handed-down as basic therapeutic agents for analgesic, antispasmodic,

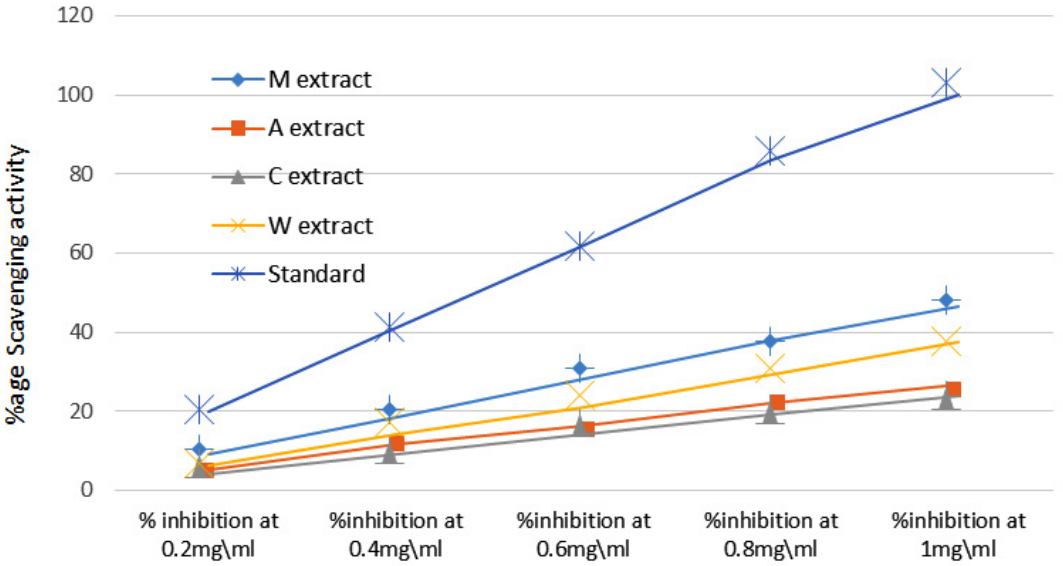

Fig. 5. Free radical scavenging activity of extracts compared to standard L-ascorbic acid at different concentrations

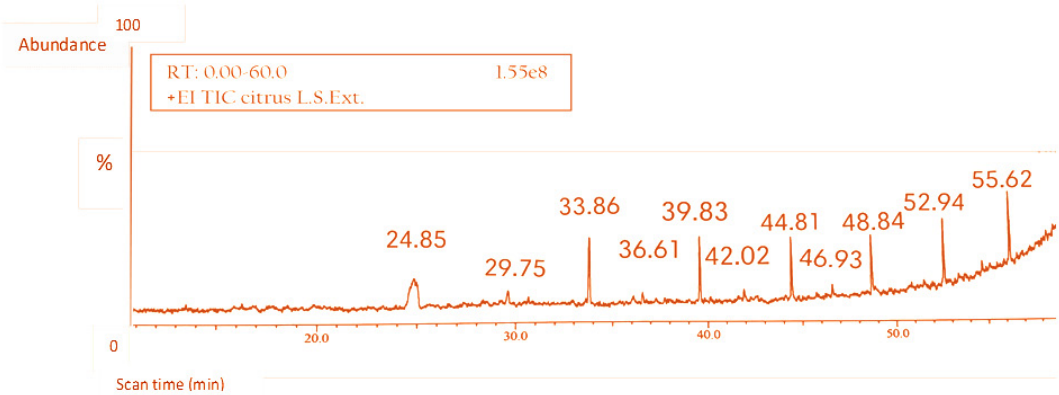

Fig. 6. A typical gas chromatogram of the chemical ingredients of methanol extract of *Citrullus lanatus* seeds

and antibacterial effects[24]. According to research, alkaloids affect the central nervous system and can act as pain relievers in some cases like morphine. The presence of alkaloids has been discovered in phytochemical screens of most plants traditionally used to treat malaria[14,25]. Saponins are found in plants

Table 5. Biologically active chemical compounds of methanol extract from *Citrullus lanatus* seeds

S. No.	Compound identified	CAS No.	Retention time	% Area	MW
1	Lupeol	000545- 47-1	24.85	8.48	426.70
2	11-Dodecen-1-yl acetate	35153-10-7	29.75	0.98	226.40
3	n- Hexadecanoic Acid	000057- 10-3	33.86	15.78	256.42
4	Cyclononasiloxane, octadecamethyl	000556- 71-8	36.61	0.29	667.39
5	Cis-7-Dodecen-1-yl acetate	014959- 86-5	39.88	17.29	226.36
6	Octadecanoic Acid	000057- 11-4	42.02	0.41	282.50
7	8-Nonenoic acid	31642-67-8	44.81	14.63	156.22
8	γ-Sitosterol	000083- 47-6	46.93	0.89	432.70
9	9, 12- Octadecadienoic acid (Z, Z), methyl ester	000112- 63-0	48.84	13.29	294.47
10	β-Sitosterol	000083- 46-5	52.94	14.16	412.70
11	11-Dodecenol	35289-31-7	55.62	15.64	184.32

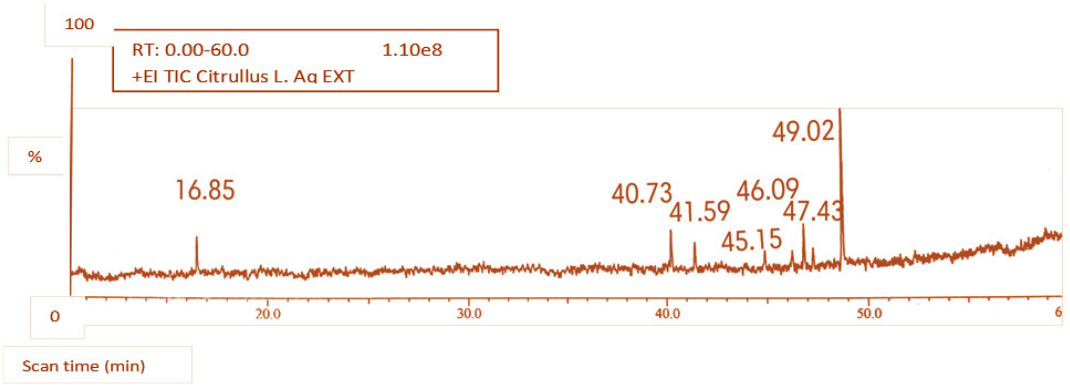

Fig. 7. A typical gas chromatogram of the chemical ingredients of aqueous extract of *Citrullus lanatus* seeds

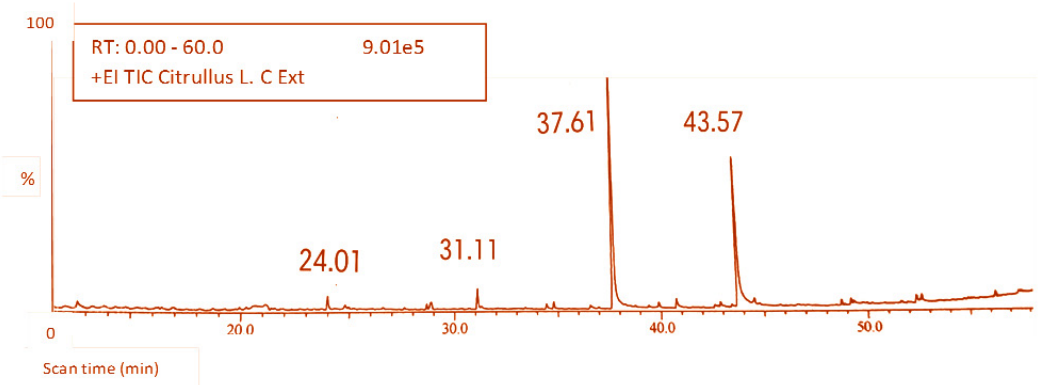

Fig. 8. A typical gas chromatogram of the chemical ingredients of chloroform extract of *Citrullus lanatus* seeds

and indicate that they have the ability to precipitate and coagulate red blood cells[11, 20]. Phytosterols are one of several nutrients that are said to be beneficial to the heart. Low-density lipoprotein (LDL) cholesterol can be reduced by roughly 10% by taking 2–3 grams of phytosterols each day for 3–4 weeks[26] According to human studies, people who consumed the most phytosterols had a lower incidence of stomach, lung, breast, and ovarian cancer[27]. Phenolic compounds have been shown to act as antioxidants with a wide range of therapeutic effects, including anticancer, anti-inflammatory, and diabetic activities. Some molecular targets of pro-inflammatory mediators in inflammatory reactions are known to be inhibited by phenolic substances such as gallotannins, condensed tannins, and flavonoids. The phytochemicals also function as antioxidants, scavenging free radicals and therefore reducing inflammation[28]. The GC-MS results showed that the seeds contain many phytochemicals with Lupeol, 9,12-octadecadienoyl chloride, and Bis(2-ethylhexyl) phthalate having the least retention times and 11-Dodecenol, Methyl tetradecanoate, and Oxazole having the highest retention times. Phytochemical analysis of the seeds showed many compounds, apparently with diverse pharmacological and biological significance. However, Phenol, 2,2-methylenebis [6-(1,1-dimethylethyl)-4-ethyl], n- Hexadecanoic Acid, Cyclopropanecarboxylic

Table 6. Biologically active chemical compounds of aqueous extract from *Citrullus lanatus* seeds

S. No.	Compound identified	CAS No.	Retention time	% Area	MW
1	(9,12- octadecadienoyl chloride)	7459-33-8	16.85	7.98	298.9
2	Oleic acid (9 octadecenoic acid)	112-79-8	40.73	9.75	282.5
3	Palmitic acid	57-10-3	41.59	5.31	256.42
4	Dioctyl ester	-	45.15	1.21	-
5	Phenol, 2,2-methylenebis [6-(1,1-dimethylethyl)-4-ethyl]	88-24-4	46.09	1.83	368.55
6	4H-Pyran-4-one,2,3-dihydro-3, 5- dihydroxy-6-methyl-	28564-83-2	47.43	8.20	144.12
7	Hexadecanoic acid, methyl ester	112-39-0	47.87	1.30	270.45
8	Methyl tetradecanoate	124-10-7	49.02	32.76	242.40

Table 7. Biologically active chemical compounds of chloroform extract from *Citrullus lanatus* seeds

S. No.	Compound identified	CAS No.	Retention time	% Area	MW
1	Bis(2-ethylhexyl) phthalate	0017-81-7	24.01	0.92	390.60
2	Nonivamide	002444- 46-4	31.11	1.87	293.4
3	Cyclohexene, 1-pentyl-4-(4-propylcyclohexyl)-	108067- 17-0	37.61	36.87	276.5
4	2- Chloroethyl linoleate	025525- 76-2 -	43.57	33.64	342.90

Table 8. Biologically active chemical compounds of acetone extract from Citrullus lanatus seeds

S. No.	Compound identified	CAS No.	Retention time	% Area	MW
1	Cyclopropanecarboxylic acid	1759-53-1	39.91	1.76	86.09
2	1-Cyclohexyl-1-propyne	17715-00-3	42.72	17.86	122.21
3	1-Azabicyclo(3,1,0) hexane	73799-64-1	43.91	34.09	83.13
4	Oxazole	288-42-6	58.70	2.19	69.06

acid like phytochemicals present in the seeds are known for antioxidant with anti-bacterial properties[29]. The phytochemical profile of the watermelon seed indicated the presence of various bioactive compounds which can be utilized for medicinal purposes. The identified compounds have several biological properties with a potential pharmacological activity that play a central role in traditional medicines and also as cosmetics commercial commodities in the markets[30]. Literature indicated that plants are the backbone of traditional medicine, and the medicinal activity of plant extract is due to different bioactive compounds in the extract with potential bioactive compounds. The activities of watermelon seed constituents such as Dodecenol, 11-Dodecenol, f" -Sitosterol, 1-Azabicyclo (3,1,0) hexane, Cyclopropanecarboxylic acid, Nonivamide, 8-Nonenoic acid, g" - Sitosterol have been revealed to possess antimicrobial, antioxidant, anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, antiarthritic, antihistaminic, antieczemic, immunomodulatory and cardioprotective activities. Oxazole has anti-cancerous, anti-viral, anti-diabetic, and antibiotic activity [31]. V.H.A. Enemor et al., 2019[32] revealed the presence of many other benign chemical compounds in *Citrullus lanatus* seeds beyond the retention time of 60 mins. Some noteworthy such compounds are Pyrrole, Isoxazole, Methyl Guanidine, 2-Propenenitrile, Thiirane, Methanesulfonyl fluoride, Silamine, Oxirane carboxaldehyde, 1,6-dichloropyruvic acid, 3-Methyl-1,3-pentadiene, Propiolamide, N-Ethylformamide, Fluoramine, and Fomepizole. These are also pharmacologically active compounds found in *Citrullus lanatus*. Proximate components, vitamins, amino acids, and phytochemicals readily alter depending upon the type of fruit cultivar, geographical location, climatic conditions, etc. so, results cannot be accurately compared. Because the risk of infection by antibiotic-resistant microorganisms is increasing dramatically, the identification and search for chemicals with antimicrobial action has become more important in recent years. *Citrullus lanatus* seeds show antibacterial action against many strains, according to our research, which might be attributable to

Table 9. Antimicrobial activity of plant extracts against seven microorganisms

Test strain	Zone of inhibition (mm) Methanol extract	Water extract	Chloroform extract	Acetone extract
Bacillus cereus 10451	[9.5 ± 0.7]	[10.7 ± 1.1]	[N]	[N]
S. aureus ATCC29213	[8.3 ± 1.2]	[N]	[N]	[6.9 ± 0.9]
Escherichia coli GIM1.708	[12.1 ± 1.2]	[9.2 ± 0.8]	[9.4 ± 1.3]	[N]
Escherichia coli DH5-Alpha	[N]	[8.3 ± 1.2]	[N]	[8.6 ± 0.9]
*Salmonella enteritidis*10982 (SE).	[7.3 ± 1.2]	[N]	[N]	[7.1 ± 1.3]

Values in triplicate determination (n=3) ± standard deviations, N- no zone of inhibition, S- Sample 1, S2- Sample 2

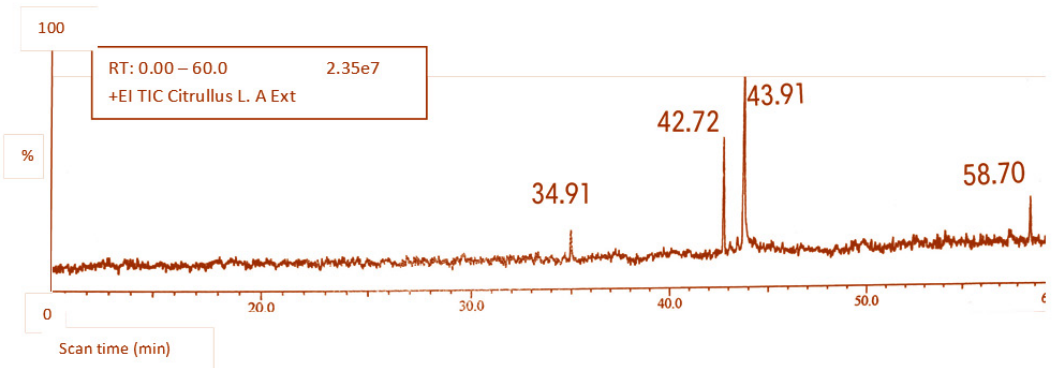

Fig. 9. A typical gas chromatogram of the chemical ingredients of acetone extracts of *Citrullus lanatus* seeds

terpenoids and phenols. *Citrullus lanatus* seeds, it may be established, have great therapeutic potential.

CONCLUSION

In this study, these seeds, which are typically thought of as a waste product of the fruit, were found to be an excellent source of physiologically important phytochemicals. Alkaloids, flavonoids, phenols, steroids, tannins, saponins, phytosterols, terpenoids, and glycosides were found in a qualitative phytochemical examination of these seeds utilizing several solvents of varying polarity and established techniques of analysis. Bioactive compounds revealed quantitively in GC-MS analysis have been revealed to be physiologically significant and essential from a pharmaceutical standpoint by an ample amount of research. Seed extracts showed substantial antioxidant and anti-bacterial action due to these phenolic and polyphenolic components. As a result, it is recommended that these compounds be analyzed as potential therapeutic agents in the management of oxidative stress-related disorders, infectious diseases, and other ailments that have taken a toll on human health.

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Conflict of Interest

There were no commercial or financial links that may be deemed a potential conflict of interest during the research.

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