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Study Of Active Constituents From Methanolic Extract Of Watermelon Fruit Extract; A Comparative Analysis Of Hybrid And Wild Cultivar.

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ABSTRACT

Plants are rich in active bio-molecules, and these molecules synthesized as secondary metabolites for a wide range of physiological functions, including defense and food reserve. These plant phytochemicals belong to a wide range of categories, including steroids, alkaloids, phenolic compounds, terpenes, saponin, and resin, etc. Watermelon is fruit for the richest source of various bio-active compounds, including vitamins and minerals. Over a period of time numerous hybrid cultivars were designed for commercial and large scale cultivation of watermelon. The key question arises among scientific platform for the active phytochemicals present in the watermelon hybrid cultivar over wild type. The present study was aimed to carry out a comparative analysis of the presence of various active biomolecules in watermelon *Citrulus Lanatus* hybrid and wild type. We have extracted active phytochemicals using multiple solvents, including methanol, acetyl acetate, and chloroform. The methanolic extract was further characterized for active phytochemicals using GC-MS for both hybrid and wild type cultivar. Based on phytochemical analysis and preliminary activity analysis, i.e., the antimicrobial and antioxidant methanolic extract does possess a comparatively higher biological activity and hence active biomolecules. The GC-MS analysis shows a wide range of phytochemicals, including Alkaloids, Phenolic compounds, Saponin, Quinone, and Coumarin in methanolic extract. On the contrary, methanolic extract of wild type cultivar does possess flavonoid, saponnin and steroid, phenolic acids and glyco-conjugates, flavonoids and glyco-conjugates, metabolites of phenylpropanoid pathway, such as gingerol, cinnamic acid, p-coumaric acid, etc.; organic acids such as citric acid, iso-citrate, fumarate, etc.; carotenoid such as beta-carotene metabolism products, curcuminoids; curcubitacin E, monosaccharaides and oligosaccharides, amino acids such as Citrulline, saturated mono and polyunsaturated fatty acids, and compound lipids such as Ceramide and Sphinganine; nucleotide bases such as guanosine, uridine, etc. The study shows watermelon hybrid cultivar are rich in diverse phytochemical over wild type cultivar precisely methanolic extract.

Keyword; GCMS, Watermelon, phytochemicals, extract methanol and biomolecules, Hybrid and wild type cultivar.

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INTRODUCTION

The plants are rich sources of bioactive molecules produced as secondary metabolites. These bioactive molecules offer a wide range of therapeutic and prophylactic potential and remain part of traditional medicine since the ancient time [1]. The fruits such as watermelon, are one of the richest sources of vitamins, minerals, essential pigments along with other critical active metabolites. The watermelon species may differ for active secondary metabolites in their nature and extent as well. The nutritional values a fruit, including watermelon, can be explored by characterizing active ingredients [2]. The molecular profiling of secondary plant metabolites provides ease in understanding its nutritional value. The molecular and cutting edge analytical characterization of such active secondary metabolites provides vital information of fruits such as antioxidant, anti-inflammatory, and antimicrobial property apart from nutritional values [3]. Plant secondary metabolites remain active ingredients in traditional medicine for many centuries. These active ingredients were used in folk medicine as plant/herb leaves, bark, powder, and root in various forms, including extract and tincture, etc. [4]. There is growing scientific literature and research in understanding the prophylactic and therapeutic use of different plant secondary metabolites [5]. The extraction of these commercially essential molecules is a complex phenomenon and depends on affinity with the solvent. A wide range of extraction techniques and solutions were evaluated for the effective isolation of these molecules.

Watermelon is a member of family *Cucurbitaceae* and closely associated with cucumber, pumpkin, squash, and gourds. The plant is native of dry climate (a native of Kalahari Desert of Africa); however, on commercial-scale plants is grown widely across the globe. Both native and genetically modified species of watermelon are raised as part of modern agriculture [6]. The fruit is rich in essential nutrients, and as per recent findings, fruit contains 92% water, 7.5% sugar/carbohydrates, and 0.4% dietary fibers. The consumption of 100 gm of watermelon provides nearly 30 Kcal. Research findings have also demonstrated that watermelon is rich in several bioactive ingredients with a vital role in performing various metabolic functions like growth, development, and protective mechanism against physiological threats [7]. The grown demand for watermelon is fulfilled by a transgenic breed of plant and commercial means of agriculture. At present, China is leading a watermelon producer on world map fulfill 20% of world need. China is also leading in not only large scale cultivation but also farming a wide range of watermelon plants [8]. Other countries such as Turkey, the United States, Iran, Republics of Korea, and India are capable of farming native and transgenic watermelon crops. The nature and diversity of active phytochemicals present in watermelon depend on habitat as well. The genetically engineered species of watermelon often contain a wide range of active phytochemicals compare to wild type cultivar.

Over some time, several studies have been carried out to extract various bioactive molecules from watermelon fruit and seed. Different solvents, including both organic and inorganic, were used to extract these molecules from the fruit, including seed. Higher yield in methanolic extract precisely in the case of hybrid watermelon signifies a wide range of phytochemicals. This might leads to the higher and diverse activity of methanolic extract compare to others. ***Ilahy et al., 2019*** have carried out a comparative analysis of active phytochemicals present in tomato and watermelon [9]. The study provides a detailed analysis of the functional quality of both the fruits based on the active phytochemical present. The research shows organic extract possesses a diverse and higher concentration of active phytochemicals in both the fruits. The study in **2001 by Adawy *et al.,*** showed a pattern of active phytochemicals in watermelon species and other similar species as well [10]. **Nagal *et al.,* 2012** studies extensively the presence of active phytochemicals in India species of watermelon [11]. The study concludes that the Indian cultivar of watermelon is rich in lycopene and phenolic compounds. GCMS provides an ideal platform for the identification and characterization of individual compounds. The high throughput studies using cutting edge techniques provide not only qualitative but also quantitative analysis of active molecules. **Pantelidis et al., 2007** carried out a comparative analysis of active antioxidant compounds in raspberries, blackberries, red currants, gooseberries, and cornelian cherries [12]. The aim of the study was to extract active metabolites and analytical characterization using GCMS.

MATERIALS AND METHODS

All the consumables and chemicals used in the present work were purchased from Sigma Aldrich India and Hi-Media India of research-grade. During the study, standard microbiological and molecular protocols were followed. The bacterial species used for the study were stored at -40^{0}C. The conventional media and growth parameters have opted during the entire study. For the extraction of active fruit secondary metabolites from watermelon, two species were used i.e., *Citrulus Lanatus* (Indian watermelon) and *Citrullus lanatus* (Chinese watermelon). Three different solvent systems with an increasing concentration of solvents were used in the study for the extraction process. For GCMS studies, methanolic extracts of watermelon from both the cultivar were used. The GC-MS (GCMSQP2010, SHIMADZU) analysis of phytochemicals from the methanolic extract of wild type and hybrid watermelon fruit was carried out [13, 14].

Extraction and Phytochemical analysis

To extract active phytochemicals fresh and fully grown watermelon fruits from both species, i.e., *Citrulus Lanatus* (Indian watermelon wild) and *Citrullus lanatus* (Chinese watermelon hybrid), were used. The fruit peel was dried to a powder and grinded for the extraction process. Using soxhlet extraction with multiple solvents i.e., ethyl acetate, methanol, and chloroform, active molecules from dried watermelon powder were extracted from both the species. The later solvent was removed by drying from extracted phytochemicals and subjected to phytochemical analysis. The extraction efficiency was determined by calculating the ratio of the weight of extract and weight of the solvent. Phytochemical analysis and characterization provide a detailed analysis of such compounds in the fruit. The crude extract of fruit collected in soxhlet apparatus using solvent system Methanol, Ethyl Acetate and Chloroform was subjected to phytochemical analysis with several biochemical tests including, alkaloid Test (Dragendroff's), flavonoid test (Shinoda test), saponin test (foam test), Quinone test, tannin test, terpenoids and steroids, phenol test, Coumarin and test for glycosides. All the phytochemical tests were performed using standard protocols [16].

Characterization of the methanolic extract by GCMS

The methanolic extract of a watermelon, both the cultivar hybrid and wild type were analyzed for phytochemical using Gas Chromatography-Mass Spectroscopy (GCMS). The GC-MS (GCMSQP2010, SHIMADZU) analysis of phytochemicals from the methanolic extract of wild type and hybrid watermelon fruit was carried out as per standard protocol [17]. Methanolic extracts containing active phytochemicals from both the cultivar of watermelon, i.e., hybrid and wild type, were dried and subjected to individual molecule characterization. We used here 1 µl of ice-cold methanolic extract into the injection chamber of GC with carrier gas flow rate 1ml/min. Here we used pure helium gas (99.995%) as the carrier gas. The temperature, in the beginning, was set 35-120^{0}C with an increasing rate of 5^{0}C/min with a holding time of 12 min. The sample was injected when the temperature reaches 250^{0}C. The phytochemicals present in both the extract, i.e., methanolic extract of wild type and hybrid watermelon fruit expressed as a percentage of the peak in the GC chromatogram [18]. The mass spectroscope connected with GC recorded on individual phytochemical based on mass and relative abundance. The GC-MS data were analyzed with software Replib and Mainlab applications [19]. The GC-MS recorded a large number of peaks along with noise, and major phytochemicals were depicted using relative mass and abundance. The results were compared with the database and previous studies. The compounds with higher prevalence and hits were crossed checked with PubChem database for chemical validation.

RESULTS AND DISCUSSION

Extraction and Phytochemical analysis

The watermelon, both the cultivar phytochemicals were extracted using methanol, chloroform, and ethyl acetate. We have reported a significant extraction yield for the methanolic extract from both the watermelon cultivar. The maximum extract yield with methanol in both the species of watermelon Indian (wild) and Chinese (hybrid), i.e., 36.5% and 40.25%, respectively, were reported. We report here higher extraction yield in a hybrid cultivar of watermelon over wild type in methanolic extract. The phytochemical analysis of a methanolic extract of a watermelon shows the presence of Alkaloids, Phenolic compounds, Saponin, Quinone, and Coumarin in the hybrid cultivar and flavonoid, saponin, and steroid in wild type cultivar. Earlier, **Hind et al., 2017** reported methanolic extract of watermelon with higher yield over another solvent in Indian watermelon species. In our previous study reported a methanolic extract of both the cultivar possesses significantly higher antibacterial and antioxidant activity [20]. We also reported in our previous finding methanolic extract of hybrid cultivar activity profile was much higher than wild type. These findings clearly have shown active phytochemicals present in watermelon possess a higher affinity for methanol over another solvent. Considering these findings, methanolic extract was further profiled and characterized using GCMS studies.

Characterization of the methanolic extract by GCMS

The GC-MS chromatogram peak report of the methanolic extract of hybrid watermelon fruit is shown in figure 1. As a result, shown here based on retention time and abundance, 40 different major phytochemicals were reported. The abundance as % of compound in the sample was a key parameter in listing major phytochemicals in both hybrid and wild type methanolic watermelon fruit extract. In the case of hybrid watermelon, methanolic extract aromatic compounds with nitrogen bases were present in a large amount [18]. However, various organic acids, such as Acetic acid and corresponding alcohols, were also reported. The aliphatic short-chain compounds such as butane and derivatives were also reported in methanolic watermelon extract. This finding suggests that being polar solvent methanol served as an ideal for the extraction of active phytochemicals from watermelon fruit in hybrid and wild type cultivar [19]. The diverse phytochemicals in watermelon fruit in hybrid cultivar demonstrates a wide range of therapeutic benefits. On the contrary, wild cultivar also contains a variety of active phytochemicals such as polyphenols, lycopene (precursor and intermediate), and aromatic nitrogenous molecules.

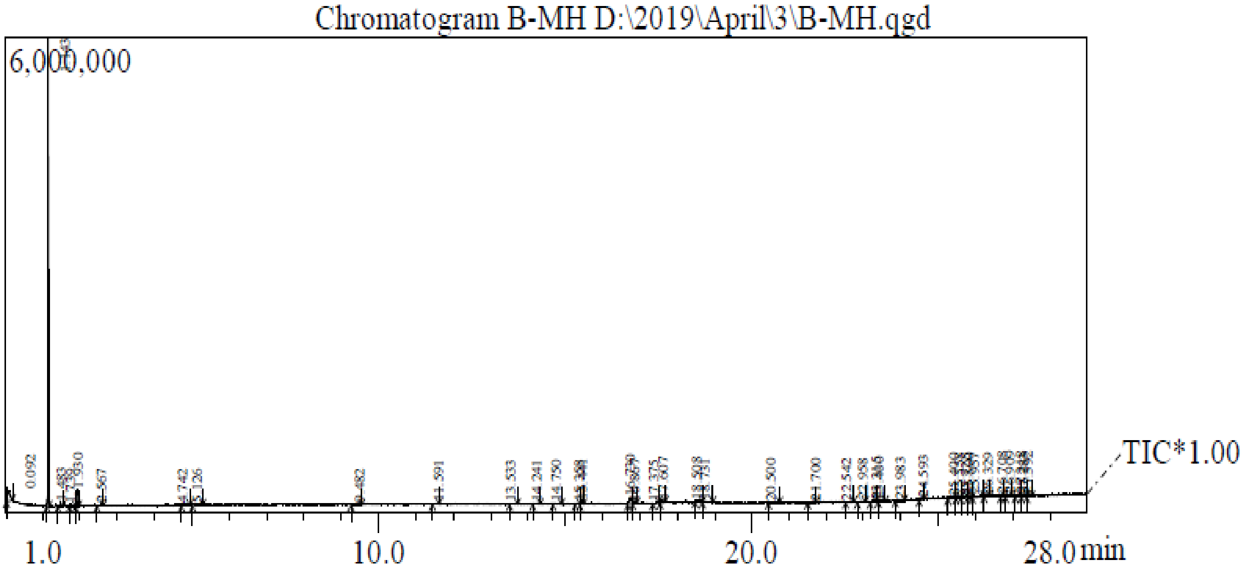

Figure 1: The figure demonstrates GC chromatogram of methanolic extract of hybrid watermelon fruit with major peaks of phytochemicals present in sample.

Table 1: Table summarizes major phytochemicals present in methanolic extract of hybrid watermelon fruit.

Name of Compound	Molecular formula	Molecular Weight
Oxiranecarboxamide, 2-ethyl-3-propyl- (CAS) Oxanamide	C8 H15 N O2	157
:3-Trifluoroacetoxypentadecane	C17H31F3O2	324
3-Trifluoroacetoxydodecane	C14H25F3O2	282
(E)-2-Nonenal $$ trans-2-Nonen-1-al	C9 H16 O	140
Monofluoroethane	C2 H5 F	48
1,3-Dioxolane-2-acetic acid, 2-methyl	C6H10O4	146
Ethanol, 2-(2-butoxyethoxy)-, acetate	C10H20O4	204
3,3-Dimethoxy-2-butanone	C6H12O3	132
CompName:4-Nitro-3-oxobutyric acid, methyl ester	C5H7NO5	161
1-METHYL-5-TRIDEUTEROMETHYLTETRAZOLE	C3 H3 D3 N4	98

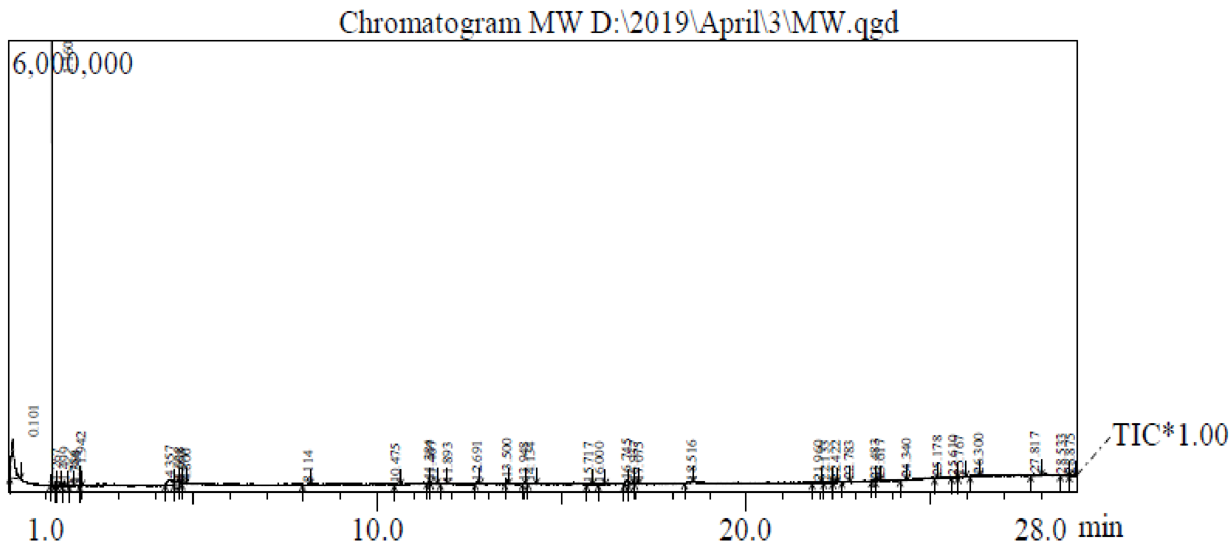

Figure 2; The figure demonstrates GC chromatogram of methanolic extract of wild watermelon fruit with major peaks of phytochemicals present in the sample.

Table 2; Table summarizes major phytochemicals present in the methanolic extract of wild type watermelon fruit.

Name of Compound	Molecular formula	Molecular Weight
1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (CAS) Bis(2-ethylhexyl) phthalate	C24 H38 O4	390
Octane, 4-chloro- (CAS) 4-Chlorooctane	C3 H3 D3 N4	98
1,3-Dioxolane, 2,2,4-trimethyl- (CAS) 2,2,4-Trimethyl-1,3-dioxolane	C6 H12 O2	116
1-Hexene, 2,5,5-trimethyl- (CAS)	C9 H18	126
Eicosane, 2-methyl- (CAS) 2-Methyleicosane	C21 H44	296
Propane, 2,2-dimethoxy- (CAS) 2,2-Dimethoxypropane	C5 H12 O2	104
1,3-Dioxolane, 2-ethyl-2-methyl- (CAS) 2-Methyl-2-ethyldioxolane	C6 H12 O2	116
1,3-Dioxolane-2-acetic acid, 2-methyl	C6H10O4	146
1,2,3-Propanetriol (CAS) Glycerol	C3 H8 O3	92
1-Hexanol, 4-methyl-, acetate	C9H18O2	158

It is the affinity of active phytochemicals towards solvent that provide ease in the extraction of these molecules. The major phytochemicals reported from hybrid watermelon methanolic extract include phenolic acids and glycoconjugates, flavonoids and glycoconjugates, metabolites of the phenylpropanoid pathway, such as gingerol, cinnamic acid, p-coumaric acid, etc.; organic acids such as citric acid, iso-citrate, fumarate, etc.; carotenoids such as beta-carotene metabolism products, curcuminoids; curcubitacin E, monosaccharaides and oligosaccharides, amino acids such as Citrulline, saturated mono and polyunsaturated fatty acids, and compound lipids such as Ceramide and Sphinganine; nucleotide bases such as guanosine, uridine, etc. [16]. The study supports that consumption of watermelon has a beneficial effect on blood pressure, renal and cardiovascular system. It also improves lipid balance and tissue fluid mineral homeostasis. There are several intermediate and secondary metabolites present in watermelon useful in diabetes management. Lycopene is a major phytochemicals present in fruits like watermelon and ripe tomato exhibit antioxidant activity [20]. There are long-chain unsaturated aliphatic compounds that have a complex synthesis pathway. In GCMS study, we reported a series of intermediate compounds as precursor and or intermediate of lycopene biosynthesis such as 4-Methoxycarbonylmethylundec-3-enedioic acid, dimethyl ester, and Acetic acid, 10,11-dihydroxy-3,7,11-trimethyl-dodeca-2,6-dienyl ester, etc.

On the contrary, wild type cultivar of watermelon fruit possesses less diverse phytochemicals to compare to hybrid cultivar. As the results are shown in figure 2, wild type watermelon methanolic extract GC Chromatogram and phytochemicals (Table 2) major phytochemicals are phenolic compounds, metabolites, and derivatives. Further, the methanolic extract also contains unsaturated aliphatic as key precursor for lycopene and several other acids. The GCMS analysis have shown a wide range of active phytochemicals in wild type methanolic extract of watermelon including curcubitacin E, curcuminol, 6-gingerol, 4'-apo-beta-psi-caroten-4'-al, apigenin 7-(4'',6''-diacetylalloside)-4'-alloside, chalconaringenin 2'-rhamnosyl-(1-4)-glucoside, Narenginin 7-O-(2'',6''-di-O-alpha-rhamnopyranosyl)-beta-glucopyranoside. These compounds were reported in hybrid watermelon methanolic extract as well [12]. The listed molecules and their metabolites are responsible for antioxidant activity of watermelon methanolic extract as potent inhibitor of DPPH. Other compounds reported in low abundance includes curcumin, catechin, docosanol, berberin, tetracosanol, quercetin, and 16-hydroxy-cleroda-3, 13-diene-16, 15-olide, (16-H), are well studies for anti-diabetes potential [21}. The wild type watermelon methanolic extract is rich in various amino acids, including amino acids such as Citrulline, L-Arginine, Histidine, Tryptophan, etc.

CONCLUSION

Plant-based phytochemicals are active in offering several therapeutic potentials and remain part of traditional medicine for many centuries. The watermelon is a classic example of fruit rich in active phytochemicals. Several varieties of watermelon are grown worldwide with varying active phytochemicals. India and China are a leader in producing watermelon, both wild type and hybrid. These varieties of watermelon offer antioxidant, antibacterial, and other therapeutic properties. The fruit pulp and peel are rich in various vital minerals and vitamins, along with a series of active secondary metabolites. The methanolic extract of watermelon in both the cultivar had shown a diverse and higher concentration of phytochemicals. The GCMS study has shown hybrid cultivar of watermelon is rich in active phytochemicals over wild type. The genetically engineering watermelon cultivar acquires unique phenotype and characteristics, including pest resistance, water-resistant, and heat resistant as well. The plant fight against these hearse conditions by producing a wide range of phytochemicals, and hence here in this study, we report phytochemical diversity is much higher in hybrid over wild type cultivar. These varieties of watermelon also offer a wide range of nutritional and therapeutic benefits.

Conflict of Interest; The author declares no conflict of interest.

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