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Phytochemical Composition, of Solanum melongena, *Solanum melongena* L. and its correlation with Bioactive Compounds.

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ABSTRACT

Solanum melongena organic products are known to contain distinctive classes of phenolic phytochemicals (flavones, phenolic acids, and anthocyanins) that can apply advantageous effects on human health. This examination created strategies for the subjective and quantitative investigation of phenolic compoundsin the peel of Solanum melongena natural products. Solanum melongena peel was removed utilizing fluid methanol before phenolic profiling with UHPLC-ESI-MS-MS. Solanum melongena peel removes yielded a profile of 17 phenolic acids, and flavones. Add up to flavonoid substance was likewise decided in methanolic extricate. Peel extracts of Solanum melongena demonstrated the most antioxidant capacity, and in addition inhibitory movement toward lipid peroxidation and reducing power. All in all, Solanum melongena peel has a vigorous profile of phenolic phytochemicals with magnificent cell reinforcement properties.

Keywords: Solanum melongena, phenolic acids, flavones and HPLC

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INTRODUCTION

Solanum melongena is one of the commonest vegetables devoured in Saudi Arabia. The unripe organic product is essentially utilized as a cooking vegetable for different dishes. Diverse assortments of Solanum melongena of various size, shape and shading are accessible in the market. The most broadly developed assortment in Saudi Arabia is the lengthened ovoid or thin ones with a dim purple skin. Concentrate of Solanum melongena with purple skin has been appeared to have a high limit in the searching of superoxide radicals and restraint of hydroxyl radical age by chelating ferrous iron (Kaneyuki et al., 1999; Noda et al., 2000). Notwithstanding the helpful impacts related with Solanum melongena, just a constrained writing is accessible on the cancer prevention agent adequacy, particularly as for shading distinction. Solanum melongena, (Solanaceae), a standout amongst the most far reaching vegetable devoured the world over contains an assortment of phytochemicals, for example, flavonoids which give a bunch of medical advantages. Solanum melongena organic product is accounted for to be a rich wellspring of ascorbic corrosive and phenolics, both of which are capable cell reinforcements (Vinson et al., 1998). Solanum melongena separates have been accounted for to effectively smother the advancement and development of tumors, lung growth (Matsubara et al., 2005), restrain irritation (Keli et al., 1996), and cardiovascular maladies (Knekt et al., 1996 and 1997). Solanum melongena has gotten an expanded enthusiasm among buyers and analysts overall due to its medical advantages and is positioned among the best 10 vegetables as far as cancer prevention agent limit (Cao et al., 1996).

Hydroxycinnamic corrosive conjugates are the primary class of phenolics display in Solanum melongena of these, chlorogenic corrosive (5-O-caffeoylquinic corrosive and its isomers) normally represents 70-95% of aggregate phenolics in Solanum melongena tissue (Whitaker and Stommel, 2003). The valuable impacts chlorogenic corrosive and related mixes introduce in minor amounts in Solanum melongena are various. Moreover, they are accounted for to bestow hostile to tumoral exercises (Sawa et al., 1998; Triantis etal., 2005).

The present examination was done to assess and look at the *in vitro*evaluation he biochemical analyses of Solanum melongena concerning peel color.

MATERIALS AND METHODS

Chemicals and apparatus

All chemicals and reagents were of analytical grade and were purchashed from Sigma Chemical Co. (St. Louis, MQ, USA), Aldrich Chemical Co. (Steinheim, Germany) and Alfa Aesar (Karlsruhe, Germany). All spectrophotometric measurements were performed on UV–VIS spectrophotometer MA9523-SPEKOL 211 (ISKRA, Horjul, Slovenia).

Plant material

Two of the most popular hybrid varieties of Solanum melongena (*Solanum melongena L.*) white and black were obtained from a local market in Jeddah, Saudi Arabia. The peel was manually removed and immediately frozen in liquid nitrogen, comminuted to obtain a fine powder and stored frozen at D80 °C until analyses (Shahiladevi and Jegadeesan 2017).

Preparation of the extracts

Exactly 5 g of lyophilized peel were broken into small pieces using a cylindrical crusher, and extracted with methanol (99.8%), using a Soxhlet apparatus.Extracts were then filtered through a Buchner funnel with a filter paper (Schleicher &Schuell, Dassel, Germany). The obtained filtrate was concentrated in vacuo at 28 °C to dryness. The residues were stored in black glass bottle for further analysis (Shahiladevi and Jegadeesan 2017).

Determination of flavonoid content

The total flavonoid content was determined according to Brighente et al. (2007). 0.5 ml of 2% aluminium chloride ($AlCl_3$) in methanol was mixed with the same volume of methanol solution of plant

extracts. After 1 h of staying at room temperature, the absorbance of the samples were measured at 415 nm on a spectrophotometer versus blank sample. Total flavonoids were determined as rutin equivalents (mg RU/g dry extract), and the values are presented as means of triplicate analyses.

Determination of total antioxidant capacity

The total antioxidant activity of the methanol extracts were evaluated by the phosphor molybdenum method (Prieto et al. 1999). The assay is based on the reduction of Mo (VI) – Mo (V) by the antioxidant compounds and subsequent formation of a green phosphate/Mo (V) complex at acid pH. 0.3 ml of sample extracts were combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mm ammoniummolybdate). The tubes containing the reaction solution were incubated at 95°C for 90 min. Then the absorbance of the solution was measured at 695 nm using spectrophotometer against blank after cooling to room temperature.

Methanol (0.3 ml) in the place of extract was used as the blank. Ascorbic acid (AA) was used as standard and the total antioxidant capacity is expressed as milligrams of ascorbic acid per gram of the dry extract.

Determination of the inhibitory activity toward lipid peroxidation

The antioxidant activity of the methanolic plant extracts were determined by the hiocyanate method (Hsu et al. 2008). 0.5 ml of stock solutions of the peel extracts was added to linoleic acid emulsion (2.5 ml, 40 mM, pH 7.0). The linoleic acid emulsion was prepared by mixing 0,2804 g linoleic acid, 0.2804 g Tween-20 as emulsifier in50 ml 40 mM phosphate buffer and the mixture was then homogenized. The final volume was adjusted to 5 ml with 40 mM phosphate buffer, pH 7.0. After incubation at 37°C in the black for 72 h, a 0.1 ml aliquot of the reaction solution was mixed with 4.7 ml of ethanol (75%), 0.1 ml $FeCl_2$ (20 mM) and 0.1 ml ammonium thiocyanate (30%). The absorbance of this mixture was measured at 500 nm, after it was stirred for 3 min. Ascorbic acid, andgallic acid were used as a reference compounds. To eliminate the solvent effect, the control sample, which contained the same amount of solvent added to the linoleic acid emulsion in the test sample and reference compound, was used. Inhibition percent of linoleic acid peroxidation was calculated using following formula:

$$\% \text{ inhibition} = \frac{Ac - As}{Ac} \times 100$$

Measurement of reducing power

Reducing power was determined according to the method of Oyaizu (1986). 2.5 ml of methanolic extracts was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. After 2.5 ml of 10% trichloroacetic acid(w/v) were added, the mixture was centrifuged at 1000 rpm for 8 min. The upper layer5 ml) was mixed with 1 ml of 0.1% of ferric chloride, and the absorbance was measured spectrophotometrically at 700 nm. A higher absorbance of this mixture indicates a higher reducing activity. AA was used as standards.

Analysis of phenolic acids through HPLC

Phenolics were identified by high performance liquid chromatography coupled with tendem mass spectrometry (HPLC/MS/MS). The HPLC system (Shimadzu Co., 10VP Series, Columbia, MD, USA) employed a Hypersil Gold C18 (3 lm particle size; 150mm length 3.0 mm ID; Thermo Electron Co., Bellefonte, PA). Five microlitres of the peel extract was injected onto the column and a gradient elution was used for separations (Niño-Medina et al. 2017). Solvent A consisted of 10% methanol in H_2O adjusted to pH 3.5 with formic acid. Solvent B consisted of 20% H_2O (pH 3.5 with formic acid), 20% MeOH, and 60% acetonitrile. At a flow rate of 0.3 ml/min, the following linear gradient was used: 0 min, 100% A; 10 min 20% A; 20 min, 40% A; 40 min, 0 % A; held at 0% A for 15 min. Five minutes of equilibration at 100% A was allowed before and after each injection. Effluent from the column was introduced into a tendem mass spectrometer (triple-quadrupole, Micromass, Inc., Beverly, MA, USA) equipped with a pneumatically-assisted electrospray ionisation source

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(ESI). Mass spectra were acquired in the negative ion mode under the following parameters: capillary voltage, 3 kV; source block temperature, 120°C; desolvation gas temperature, 400°C. Nitrogen was used as the drying and nebulizing gas at flow rates of approximately 50 and 450 l/h. For full-scan HPLC–ESI–MS analysis, spectra were scanned in the range of 50 to 1200 m/z. Data acquisition and processing were performed using a Mass-Lynx NT 3.5 data system (Micromass Inc., Beverly, MA, USA).

RESULTS AND DISCUSSION

Flavonoid contents

The flavonoid content of black and whiteSolanum melongena ranged from 4.2 to 6.1mgR/gdw. It appears that the flavonoid content of white higher than flavonoid of the black. Conversely white apparently increases the flavonoid content of the Solanum melongena (Figures 1).These data was agreement with Dodds who found the presence of flavonoids, steroids, and alkaloids in the root as compared to the fruit extract which contains alkaloids, flavonoids, tannins, steroids, and glycosides (Dodds and Robert, 1995). The chemical detection of Solanum melongena extracts showed that it was rich in flavonoids compared to rutin as sander.

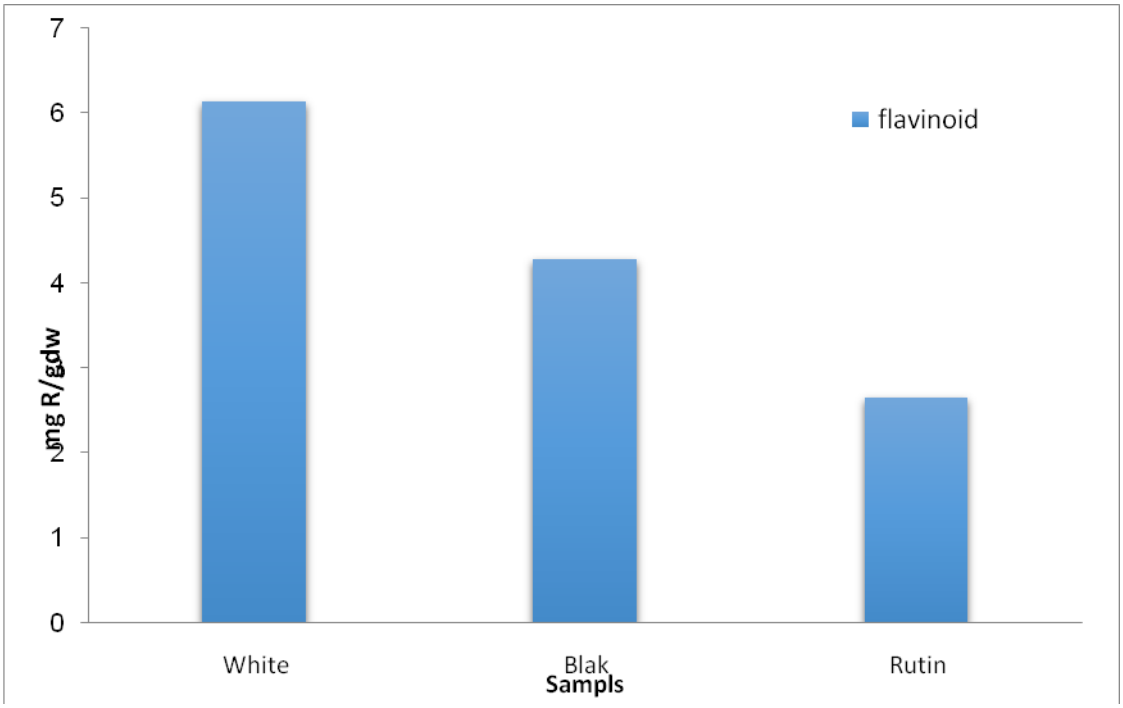

Figure 1: Total flavonoid contents of white and black Solanum melongena

Total antioxidant capacity

Figure 2 demonstrates the aggregate cell reinforcement limit property of the white and black Solanum melongena. The correlation investigations between two Solanum melongena types were performed and the outcomes show that peel parts of the Solanum melongena displays intense free radical limit capacity. Obviously, black Solanum melongena builds the free radical rummaging strength of the white.

Concentrates identified with the cell reinforcement limit of anthocyanins show that such bright mixes might be in charge of a significant part of the cell reinforcement insurance against peroxyl radicals, which make oxidative harm lipids, proteins, and nucleic acids, being vital components for the improvement of various maladies including disease (Hou et al., 2003; Wang et al., 1999).Truth be told, accessible information demonstrates that Solanum melongena are promising specialists in the administration of oxidative stress related ailments. For instance, Akanitapichat et al. (2010) watched that the cell reinforcement exercises of the Solanum melongena were related with their hepatoprotective action. In a similar vein, Das and his works

together demonstrated that there was an immediate connection between Solanum melongena s and their cardioprotective capacity (Das et al., 2011).

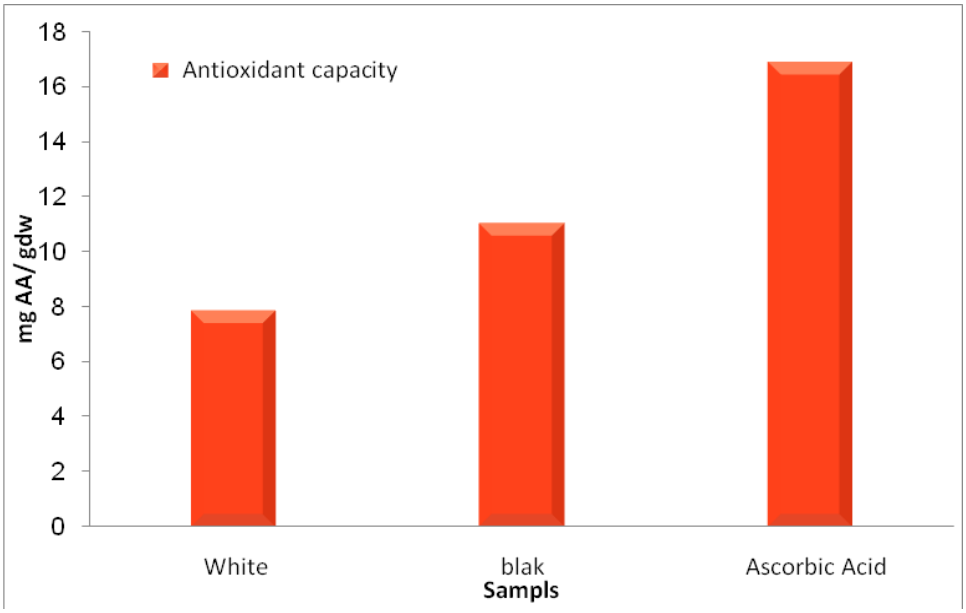

Figure 2: Total antioxidant capacityof white and black Solanum melongena

Inhibitory activity toward lipid peroxidation

Figures 3 demonstrate the impact of colure on the inhibitory impact of Solanum melongena on hepatic lipid peroxidation subjected to different oxidants ambushes. The hepatic lipids are subjected to pressure initiated peroxidation caused by Fe^{2+} within the sight of white and black Solanum melongena. So also, a similar example of results were watched when distinctive prooxidant (sodium nitroprusside) was utilized as a part of the peroxidation examine however same hepatic lipids. Actually, Figure 4 demonstrates that when hepatic lipids were subjected to oxidative worry under sodium nitroprusside attack, both white and black of Solanum melongena could fundamentally restrain the peroxidation of hepatic lipids in a manner like that watched when Fe^{2+} was utilized.

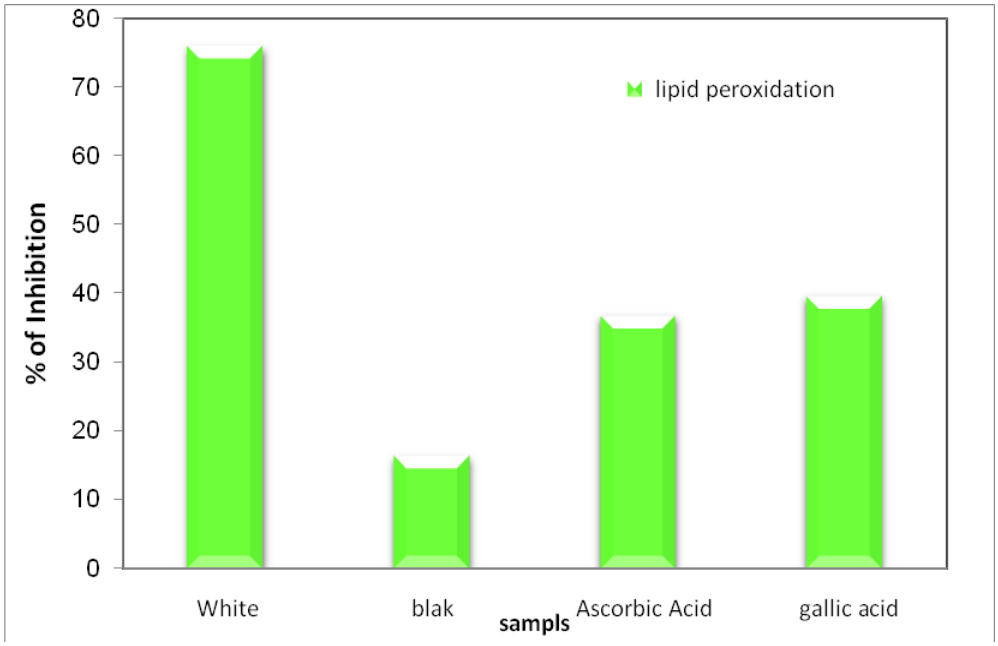

Figure 2: lipid peroxidation % of white and black Solanum melongena

The present examination showed that both white and black metabolic concentrates of solanum melongena display powerful cancer prevention agent action and clearly, the cell reinforcement properties of both Solanum melongena decidedly associate with their flavonoid substance. Our present examination corresponds with the discoveries of Sudheeseh and his collaborators who found that flavonoids disengaged from Solanum melongena demonstrated powerful cancer prevention agent action (Sudheesh et al., 1999). Theblack products of Solanum melongena may contain different mixes, for example, anthocyanins that give the natural products its trademark red shades and potentially extra cancer prevention agent impact. Truth be told, separates from purple shading little size Solanum melongena organic products with powerful cell reinforcement exercises have been ascribed to the higher phenolic and anthocyanin content (Nisha et al., 2009).

Anthocyanins are the biggest gathering of water dissolvable colors in the plant kingdom can be specifically assimilated and disseminated to the blood in people and rats after utilization of the dietary anthocyanin (Hagiwara et al., 2002; Matsumoto et al., 2001). Late investigations have demonstrated that both white and black have diminishing property action, radical searching movement on the Solanum melongena and this might be identified with the anthocyanin substance of the organic products.

The oxygen particle may deliver an exceedingly receptive oxygen animal types (ROS) by some exogenous components and endogenous metabolic procedures in human body. ROS incorporate various artificially receptive atoms, for example, hydrogen peroxide (H_2O_2), superoxide (O^{2-}), and the hydroxyl radical (•OH). The •OH in the cells can without much of a stretch cross cell films at particular destinations, respond with most biomolecules and moreover cause tissue harm and cell demise. In this way, expelling •OH is imperative for the Protection of living frameworks. The •OH searching impact by the both white and black Solanum melongena extricates, and clearly the black Solanum melongena separates display intense •OH rummaging capacity with subsequent assurance of deoxyribose harm. We may property this watched •OH searching capacity to conceivable anthocyanin substance that increments with type in the Solanum melongena.

We can conjecture that Solanum melongena concentrates may search superoxide and peroxynitrite and may additionally interface with the cyanide moiety or even the arrival of iron from the ferrocyanide moiety of SNP in this manner applying a synergistic-like cancer prevention agent impact. It is significant to say that albeit synthetic measures of cancer prevention agent action have been utilized in the present investigation, these measures don't speak to physiological oxidation occasions. Nonetheless, other *in vitro* ponders have demonstrated that Solanum melongena removes are promising competitor in the administration of diabetes and hypertension (Kwon et al., 2008) and it is in this manner trusted that *in vivo* information would emphatically relate with the saw in vitro information.

Reducing property

The reducing property of Solanum melongena is presented in Figure 4. This analysis revealed that the reducing property of the Solanum melongena for both white and black were significant compared with blank of and ascorbic acid (90. 91, and 95 %) respectively. In addition, Solanum melongena is rich in free electron and readily supplies such electron to Fe^{3+}, thereby reducing Fe^{3+} to Fe^{2+}.

The effect of Solanum melongena on the Fe^{2+} chelating properties showed that, white and black Solanum melongena have higher chelating ability. Furthermore, the chelating Fe^{2+} property was markedly enhanced. It additionally surely understood that ironFe^{3+} and iron Fe^{2+} edifices invigorate lipid peroxidation in cells (Gogvadze et al., 2003). The instrument (s) that underlies the decreasing property of both Solanum melongena extricates estimated within the sight of Fe^{2+} might be credited to the capacity of the concentrates to rummage radical, lessen Fe^{3+} and chelate Fe^{2+} and this would promptly clarify why Solanum melongena separate showed checked inhibitory impact on hepatic lipid peroxidation.

The capacity of Solanum melongena to diminish Fe^{3+} to Fe^{2+} is because of any substance ready to chelate and deactivate change metals keeps such metals from taking an interest in the start of lipid peroxidation, protein carbonylation, DNA strike and oxidative worry through metal-catalyzed responses. The capacity of the Solanum melongena to chelate change metals is along these lines thought to be because of a cancer prevention agent instrument (Guemmazet al.2018).

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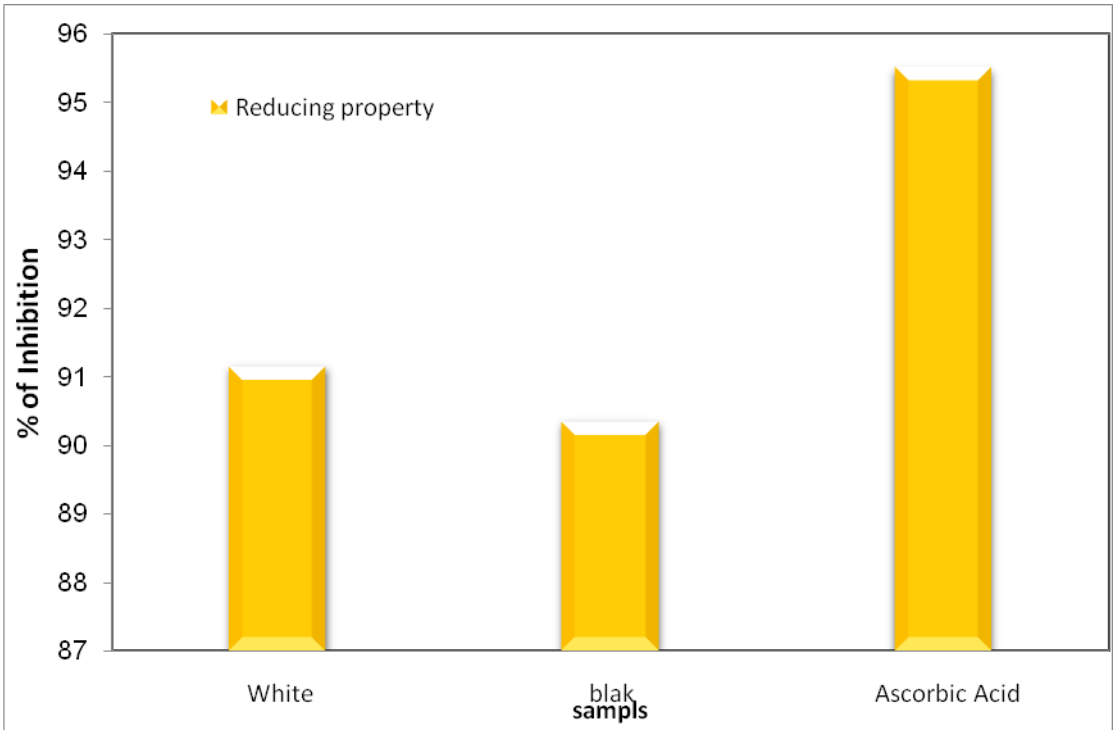

Figure 4: Total Reducing activity of white and black Solanum melongena

Phenolic acids through HPLC

The present information can construct our certainty that the ant oxidative nourishing estimations of Solanum melongena. Extraction of phenolic acids from Solanum melongena peel completed utilizing 80% methanol in water in view of past advancement think about on extraction of phenolic acids from Solanum melongena (Luthria and Mukhopadhyay, 2006). Table 1,2 demonstrates the retention time (tR), ultraviolet-visible (UV–vis) absorption maxima (kmax), and mass spectral data (molecular and major fragment ions) of the phenolic compounds extracted from the white and black Solanum melongena peel. The distinguishing proof of the diverse phenolic phytochemicals (Gallic Acid, Catechin, Coffeic Acid, Syringic Acid, Rutin, Coumaric Acid, Vanillin, Ferulic Acid, Naringenin, Querectin, Cinnamic Acid, Propyl Gallate, DihydroxyisoFlavone) were accomplished by correlation of UV– vis and mass otherworldly investigation information with those distributed in the writing. The solubility of phenolic compounds depends on the solvents used and the degree of polymerization of the phenols and the interaction of polyphenols with other compounds. For this reason, it is recommended to use methanol to extract phenols (Guemmazet al.2018)

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Table 1: Retention times (RT), ultraviolet spectral (λmax), and mass spectral data (molecular ion and the major fragment ions) of the phenolic acidsand flavonols extracted from white Solanum melongena peel

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
7	2.858	VV	0.0957	32.74913	2.0375	?
8	3.052	VV	0.0853	267.71249	16.6558	?
9	3.133		0.0000	0.00000	0.0000	Gallic acid
10	3.366	VV	0.0864	827.07111	51.4565	?
11	3.490		0.0000	0.00000	0.0000	
12	3.895	BB	0.0970	12.74261	0.7928	Catechin
13	4.886		0.0000	0.00000	0.0000	Coffeic acid
14	5.236		0.0000	0.00000	0.0000	Syringic acid
15	5.550		0.0000	0.00000	0.0000	Rutin
16	7.510		0.0000	0.00000	0.0000	Caumaric
17	7.970		0.0000	0.00000	0.0000	
18	8.274		0.0000	0.00000	0.0000	Vallin
19	8.789		0.0000	0.00000	0.0000	Ferulic acid
20	9.235	BV	0.1091	37.23352	2.3165	Naringenin
21	10.211		0.0000	0.00000	0.0000	Proygallate
22	10.464		0.0000	0.00000	0.0000	DihydroxyisoFlavone
23	10.649		0.0000	0.00000	0.0000	querctin
24	11.136		0.0000	0.00000	0.0000	Cinamic acid
25	11.349		0.0000	0.00000	0.0000	
26	14.277	BBA	0.7505	140.98854	8.7716	?
Totals :				1607.32231		

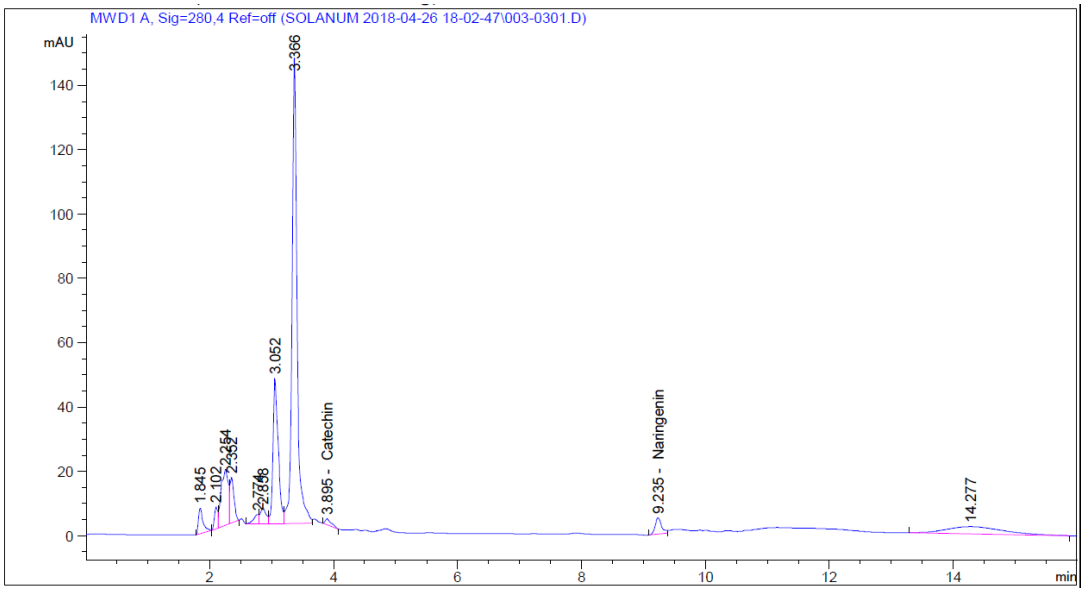

Figure 5: HPLC-DAD chromatogram of white Solanum melongena peel

The outcomes were recommended a probability of the nearness of a Naringenin, Dihydroxy isoFlavone, Syringic corrosive, Catechin derivative in black Solanum melongena. In contrast with the over two

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isomers of quinic acids, the 4-caffeoylquinic corrosive likewise demonstrated an indistinguishable atomic particle from expected yet the base particle top was at m/z 173 relating to dried out quinic corrosive (Clifford 2000). Two extra isomers of caffe oylquinic corrosive (crests 8 and 9, tR 19.829 and 20.224 min) were additionally recognized by correlation of mass and UV– vis otherworldly information (Wilson et al., 2008).

Notwithstanding the above portrayed phenolic corrosive subsidiaries, we have recognized five extra flavonol glycosides (Naringenin, DihydroxyisoFlavone, Syringic corrosive, Catechin derivative in and Rutin) in follow amounts from the black Solanum melongena. The ID of the two flavonols glycosides was affirmed by examination of HPLC maintenance time with genuine flavonols models and UV– vis and mass phantom information as beforehand revealed (Vvedenskaya et al., 2004). black seems to initiate progression in the polyphenol substance and thusly the subterranean insect oxidative possibilities of Solanum melongena removes. Be that as it may, since both white and black Solanum melongena are devoured.

Table 2: Retention times (RT), ultraviolet spectral (λmax), and mass spectral data (molecular ion and the major fragment ions) of the phenolic acids and flavones extracted from black Solanum melongena peel

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
7	3.031	BV	0.0699	1018.09314	11.5746	?
8	3.133		0.0000	0.00000	0.0000	Gallic acid
9	3.341	VB	0.1060	4541.34131	51.6301	?
10	3.490		0.0000	0.00000	0.0000	
11	3.874	BB	0.0991	38.16159	0.4339	Catechin
12	4.350	BV	0.0825	30.97882	0.3522	?
13	4.469	VB	0.0821	16.77341	0.1907	?
14	4.857	BB	0.1169	109.00497	1.2393	Coffeic acid
15	5.362	BV	0.1212	15.07750	0.1714	Syringic acid
16	5.539	VB	0.1499	28.11109	0.3196	Rutin
17	7.510		0.0000	0.00000	0.0000	Caumaric
18	7.970		0.0000	0.00000	0.0000	
19	8.274		0.0000	0.00000	0.0000	Vallin
20	8.789		0.0000	0.00000	0.0000	Ferulic acid
21	9.200	BV	0.1287	162.97951	1.8529	?
22	9.371	VV	0.0946	69.68933	0.7923	Naringenin
23	9.451	VV	0.0703	47.94920	0.5451	?
24	9.554	VV	0.1084	71.32742	0.8109	?
25	9.676	VB	0.1251	65.68336	0.7467	?
26	10.211		0.0000	0.00000	0.0000	Proygallate
27	10.349	BB	0.0716	35.84193	0.4075	DihydroxyisoFlavone
28	10.649		0.0000	0.00000	0.0000	querctin
29	11.136		0.0000	0.00000	0.0000	Cinamic acid
30	11.349		0.0000	0.00000	0.0000	
Totals :				8795.92283		

Evaluation of the six noteworthy phenolic acids is displayed in Table 2. Catechin, Coffeic acid, Syringic acid, Rutin, Naringenin and Dihydroxyiso Flavone were the significant phenolic corrosive extricated from white Solanum melongena, and it constituted around 90% of the aggregate polyphenolic mixes separated from the

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Solanum melongena peel. The amount of phenolic mixes of black Solanum melongena extricated was possibly higher than the white Solanum melongena.

These outcomes recommend a requirement for investigating extra Solanum melongena tests from different sorts to plainly assess the centrality of natural cultivating on phenolic phytochemicals content and their cancer prevention agent action in Solanum melongenas. Seventeen diverse phenolic acids were recognized in Solanum melongena peel tests, comparative with what we have already detailed in Solanum melongena peel (Singh et al 2017).

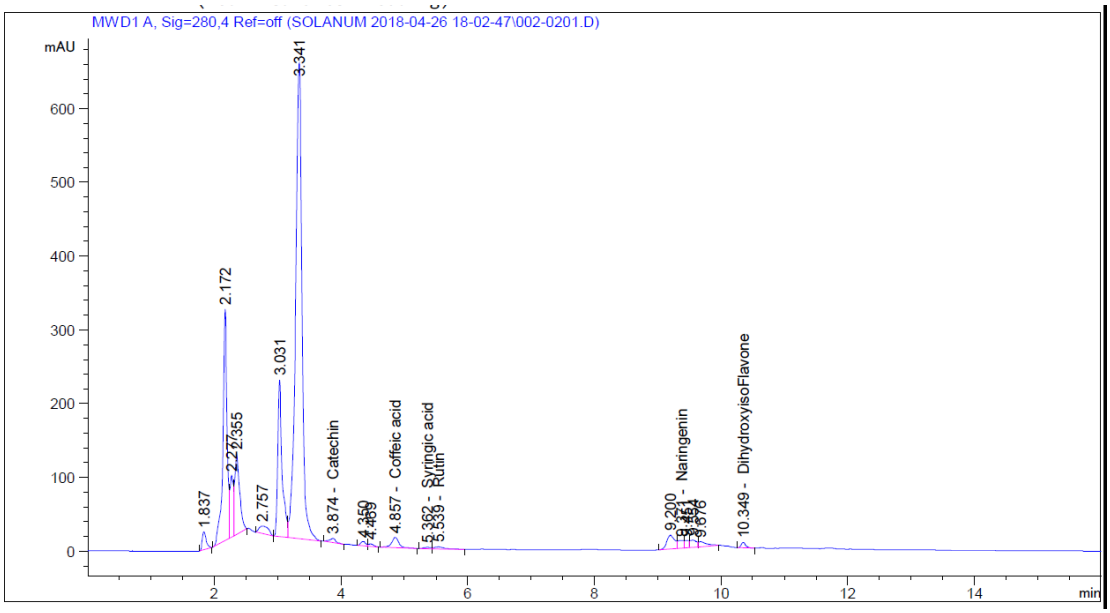

Figure 6: HPLC-DAD chromatogram of black Solanum melongena peel

In this manner, it is conceivable that natural developed Solanum melongena deliver more phenolic acids in responding to the heavier ecological anxieties experienced as a major aspect of the natural developing condition. As phenolic acids e.g. chlorogenic corrosive, caffeic corrosive and ferulic corrosive have been appeared to have different wellbeing advantageous exercises (Feng et al 2005) the natural development condition could conceivably enhance Solanum melongena skin wellbeing advancing potential.

As skin speaks to the piece of the plant with the best introduction to ecological pressure and the plant well on the way to change its phenolic articulation to keep damage from natural pressure, these outcomes show that Solanum melongenapeel and mash have distinctive phenolic appropriation/organization designs came about because of plant tissue pressure presentation. Comparative outcomes have been seen in different natural products/vegetables, for example, tomato, grape, apple, pomegranate and mango (Ribeiro et al 2008; Toor, and Savage2005).

CONCLUSION AND RECOMMENDATION

The outcomes displayed in this investigation obviously show that the total phenolic substance of two Solanum melongena cultivars (high contrast which more well-known kind KAU utilized. The routinely developed black Solanum melongena indicated possibly higher or measure up to phenolic substance and flavonoids content. Be that as it may, Solanum melongena demonstrated altogether higher inhibitory activity towered lipid peroxidation and reducing power as well as total antioxidants capacity cancer prevention agent action when contrasted with the type of Solanum melongena. The phenolic reinforcement movement of the highly contrasting cultivar corresponded well with the phenolic content.

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