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MASTREVIRUS EFFECT ON METABOLIC AND BIOLOGICAL ACTIVITIES IN TOMATO LEAVES

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ABSTRACT

This study was planned to illuminate the secondary metabolites of *Chickpea chlorotic dwarf virus* (member of mastrevirus) infected tomato plants by using phytochemical profiling procedures and their comparison with control healthy tomato plants. Metabolite profiling through GCMS indicated fourteen bioactive compounds in mastrevirus inoculated symptomatic while eight bioactive compounds were present in healthy tomato plant sample. The plant extracts were used for comparative qualitative phytochemical screening indicating the presence of alkaloid, saponins, terpenoids, tannins, phlobatannins, cardiac glycosides, anthraquinones, flavonoids and phenolic compounds in control and experimental plants. However, coumarins were absent in both samples. These metabolites were further tested for their antimicrobial potential against two gram negative bacteria *viz*., *Pseudomonas aeruginosa* and *Xanthomonas campestris* and two pathogenic ascomycetous fungal strains *Aspergillus flavus* and *Penicillium verrucosum*. Control and experimental plants showed maximum inhibition in methanol extract i.e., 19±0.57a against *P. aeruginosa* and 27±2.08a and 21±1.73ab against *X. campestris* respectively. Control plants exhibited maximum inhibition i.e., 30±1.53bc in chloroform extract and experimental plants showed maximum inhibition (35±3.6a) in distilled water extract against *P. verrucosum*. Chloroform extract of control plants and methanol extract of experimental plants showed maximum inhibitory zone (31±2.65a and 25±2.52abc) against *A. flavus*. Furthermore the control and experimental plants showed maximum percentage DPPH free radical scavenging activity in chloroform extract i.e., 0.25 mg/mL dilution. The findings of this current study elaborate our understanding about the metabolic changes in virus infected tomato plants.

Keywords: Tomato leaves, *Chickpea chlorotic dwarf virus*, Metabolite profiling, Antibacterial activity, Antifungal Activity, Antioxidant activity.

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INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is the most important vegetable crop around the globe. It is economically attractive and fast growing herbaceous cash crop with high yield potential. It is one of the conspicuous members of *Solanaceae*. Health related benefits of tomatoes are enormous primarily which includes healthy and balanced diet being the richest supply of essential amino acids, minerals, vitamins, dietary fibres and sugars. Global per annum production of fresh tomatoes has been estimated to be around hundred million tons. In Pakistan, tomato has its own importance as one of the highly earning cash crops. Generally, per annum export of tomatoes from Pakistan is estimated to be around 9832 tons in the previous five years (Khokhar and Hri, 2013). Prevention, identification, management and treatment of tomato diseases are mandatory to earn significant amount of foreign exchange and by obtaining a magnificent tomato harvest. Viral diseases can deteriorate the quality and quantity of tomato fruit. Viral diseases are considered to be greatly limiting the tomato yield. Unavailability of antiviral products, the viral control strategies either rests upon prevention of viral spread or by growing genetically resistance varieties and to some extent by eradication of diseased plants (Hanssen *et al*., 2010a).

Begomovirus belonging to family *Geminiviridae* which harbours vast diversity of viral species are commonly reported viral pathogens of tomato, due to high susceptibility of tomato to this genus. Recently, *Chickpea chlorotic dwarf virus* (CpCDV) that belongs to the genus *Mastrevirus* of the same family is found to infect the tomato plant (Zia-Ur-Rehman *et al*., 2015). In addition to that, rigorous breeding programs going on around the globe in order to improve production have narrowed genetic diversity which is imperative for viral resistance in commercially demanded cultivars (Hanssen *et al*., 2010b). Thus, it's the need of hour to characterize and identify the pathogens infecting tomato plants.

Tomato has been renowned for containing health-beneficial vitamins and antioxidant (Yuwei, 2016). Viral infections, along other various human diseases, are accompanied by oxidative stress (Gullberg *et al*., 2015; Kumar *et al*., 2009). Oxidative processes enhance virus replication in infected cells; reduce cellular proliferation which later induces cell apoptosis (Schreck *et al*., 1991). Identification of chemical classes (of secondary metabolites) exhibiting antimicrobial activity is a remarkable step towards the identification and isolation of pure chemicals (Nice, 2013). If the identified antimicrobial compounds (from this research) are suitable to be directly used as antibiotic drugs or preservatives, their chemical structures and mode of actions could lead to discovery of novel bacterial target sites to be used in future drug development. To our knowledge, metabolites and phytochemical profiling coupled with antimicrobial and antioxidant potential of CpCDV infecting tomato plants was evaluated **first time** and discussed in this research.

MATERIALS AND METHODS

Selection of infectious clones: Already prepared clone of mastrevirus namely *Chickpea chlorotic dwarf virus* (CpCDV) GenBank accession number: KP8816050 (Zia-Ur-Rehman *et al*., 2015) was selected in this study. Previously, it was checked for their infectivity in tomato plants at 4-5 leaf stage by using agroinfiltration method (Santi *et al*., 2008) as depicted in Fig. 1.

Detection of virus DNA in *S. lycopersicum* plants: For detection of infectious clones of CpCDV, the DNA of infected leaves was extracted using the method by Doyle (1991) with minor modifications and presence/absence of respective virus genome (coat protein ~738 bp and full genome ~2.6 kb) was detected by PCR by using following primer pairs:

The amplicons were subsequently cloned into TA cloning vector pTZ57R/T (InsTAclone PCR cloning kit, ThermoScientific) and sequenced in their entirety from First BASE Laboratories Sdn Bhd, Malaysia.

Sequence comparisons: All the obtained sequences were initially analysed using BLASTn with already submitted sequences available in the NCBI GenBank database (http:www.ncbi.nlm.nih.gov).

Metabolite profiling, phytochemical, antimicrobial and antioxidant activities: Comparative metabolite profiling, phytochemical test, antimicrobial and antioxidant activities of healthy tomato plants (control) and CpCDV inoculated tomato plants (experimental) were done by following the methods depicted in Fig. 2.

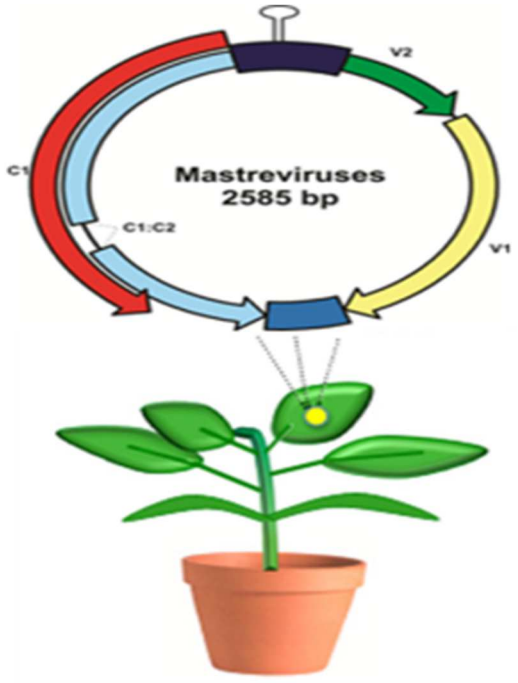


Fig. 1. Agroinfiltration of infectious clones in tomato (*Solanum lycopersicum* L.) plants.

Coat protein primers	Forward	GAGCTCAGGAATCAGAATCAGC
	Reverse	GGTACCTACTCACACAATGAAACA
Full length amplification primers	Forward	ATATTTTGATTGGAATCTGAAGTTCTTG
	Reverse	AATATGTTTTCCTTACCTACCCTAAATG

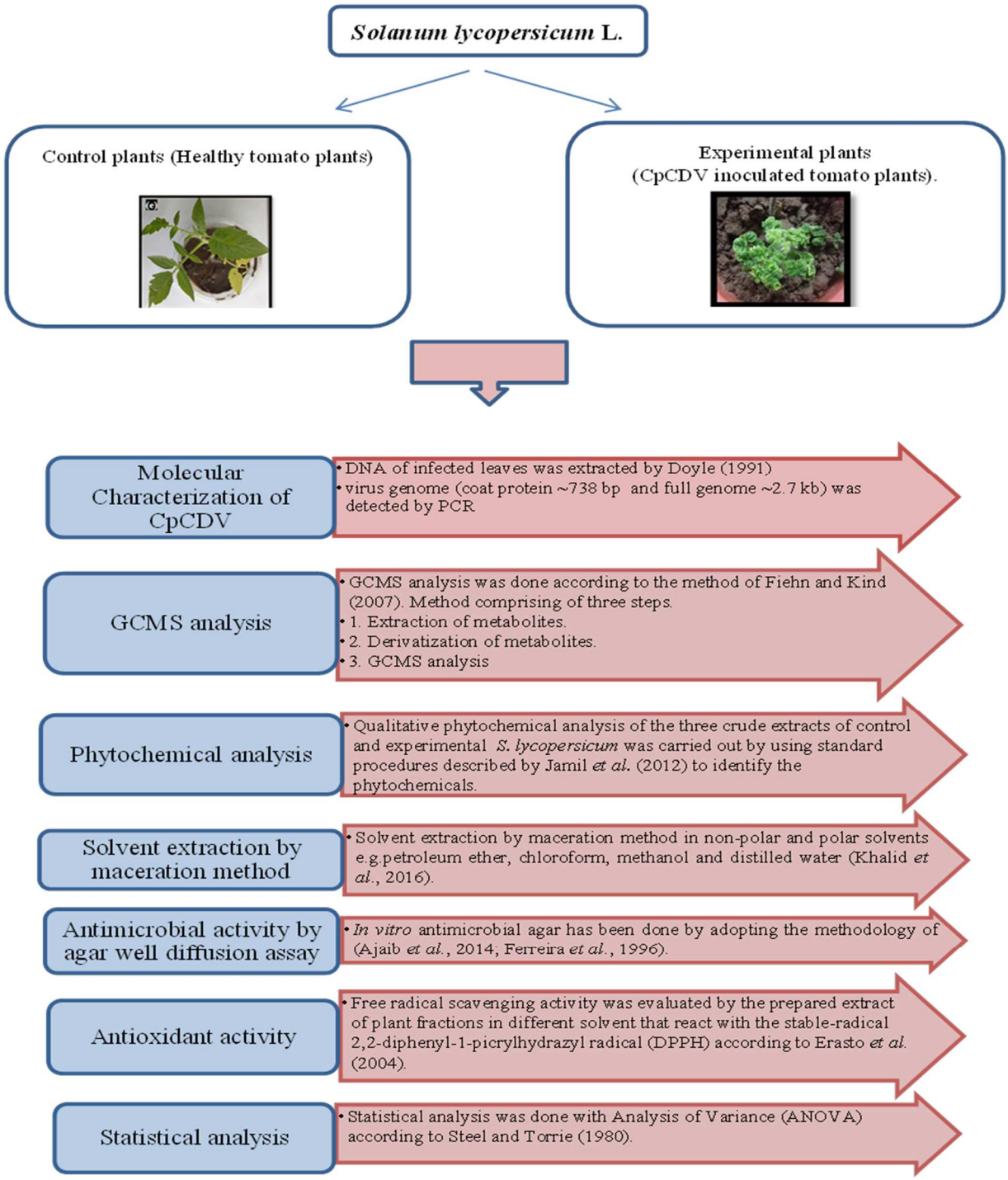


Fig. 2. Diagrammatic representation of methodology adopted in this research.

RESULTS AND DISCUSSION

Molecular characterization of Mastrevirus in infected *S. lycopersicum* plants: DNA of the infected sample has been extracted and ran on 0.8% gel using agarose gel electrophoresis. Required bands were visualized using gel documentation system. PCR was performed at Tm 56°C and coat protein gene was detected at ~738 bp while full length genome ~2.7 kb was obtained by using universal primers (Fig. 3). Geminivirus size range from 2.6-3.2 kb and coat protein gene. In tropical and sub-tropical regions of world, they are economically most important plant pathogens (Fiallo-Olivé *et al.*, 2021).

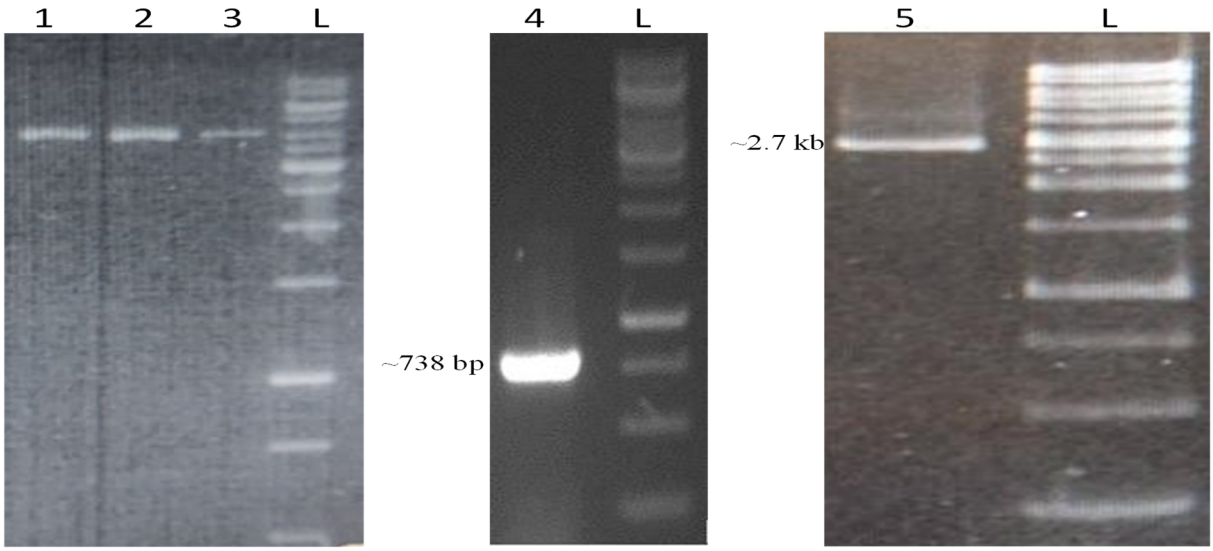


Figure 03. Illustration of (A). 1,2,3 DNA extraction from mastrevirus (CpCDV) infected tomato leaf sample (B). 4. PCR amplification of ~738 bp coat protein (CP) and (C) 5. ~2.7 kb of full length genome of the CpCDV infected samples. L stands for 1kb DNA ladder.

Comparative metabolite profiling of control and experimental tomato plants: During fermentation, microorganisms have the capability to degrade organic substances with their own enzymes due to which various remarkable metabolic changes occur. New molecular approaches are needed to gain new insights in this regard (Kim *et al*., 2019). The field of metabolomics involves holistic analysis of metabolic changes occurring in the complete set of a small compound (Koek *et al*., 2006). Metabolomic techniques have been applied to investigate and ensure presence of putative bioactive compounds in fruits like tomatoes and pineapples and in vegetables such as beet root and cucumber (Kazimierczak *et al*., 2004; Janningsmeier and McFeeters, 2015; Filannino *et al*., 2014). However, no study has been conducted about metabolic changes in geminivirus infected tomato leaves till now.

Thus, current study illuminate the identification of fourteen secondary metabolites of *Chickpea chlorotic dwarf virus* (member of mastrevirus) infected tomato plants (experimental plants) whilst eight compounds have been detected in healthy tomato leaves (control plants) through phytochemical profiling procedures and Gas chromatography mass spectrometry (GCMS) techniques as depicted in figure 4,5 and Table 1 and 2.

In the presented piece of work, it was concluded from GCMS analyses that five metabolites were same and twelve were different in experimental and control plants as shown in table 3. Geminivirus infection is assumed to be responsible for the difference in metabolites in the light of results reported by Rossouw *et al*. (2019), to identify the difference in metabolites in tomato by using ultra-high-performance liquid chromatography coupled to mass spectrometry (UHPLC-MS). As metabolites of tomatoes are directly related to taste and flavour (Bellens *et al*., 2008; Yilmaz, 2001), while differences in metabolites were found to be dependent on the LAB strain, which highlighted variation in quality. For example, the umami of tomato has been related to the content of citric acid and amino acids such as glycine, serine and glutamic acid (Zushi and Matsuzoe, 2011). Among them, glutamic acid plays a vital part as umami enhancer in tomato (Fuke and Shimizu, 1993).

Qualified evaluation of control and experimental tomato plants by phytochemical tests: Tomato is rich source of lycopenes. Carotenoids are famed due to their medicinal contribution to human health on account of their antioxidant power. Previously it has been demonstrated that carotenoids have powerful free radical scavenging activity specifically due to their potential in quenching atomic oxygen, counteracting sulfenyl radicals and stabilizing peroxyl radicals with the help of hydrophobic chain of polyene units (Terao *et al*., 2010).

S. lycopersicum control and experimental plants leaves were found to be active in the production of secondary metabolites as alkaloids,-saponins, tannins,-phlobatannins, terpenoids, cardiac glycosides, flavonoids, anthraquinones and phenolic compounds due to froth generation, precipitate formation, ring formation, showing the varying degree of positive results, hence possessed the strong defense system. Coumarins were totally absent in control and experimental plants (Table 4). Phenolic compounds are commonly considered as extensively distributed phytochemicals comprised of an aromatic ring with one or more hydroxyl groups attached.

In tomato, antioxidant phenols were reported to be flavonoids, phenolic acids and tannins. Recently, free radical scavenging activity of phenols has been found to take imperative part in human fitness (Karacabey and Mazza, 2010).

Flavonoid based derivatives were reported in every botanical family according to the nature and number of functional groups attached to the flavonoid ring (Beecher, 2003). In addition to that condensed tannins in food were synthesized by polymerization of flavanoids. Some commonly reported flavonoids in tomatoes were rutin, quercetin, naringin, kaempferol and myricetin. Contribution of carotenoids to human health is because of their antioxidant power and pro vitamin A activity. Phytochemical studies have revealed carotenoids as powerful free radical scavengers due to their capability in quenching singlet oxygen [$^1O_2$], neutralizing sulfenyl radicals and stabilizing peroxyl radicals through the hydrophobic chain of polyene units (Terao *et al*., 2010).

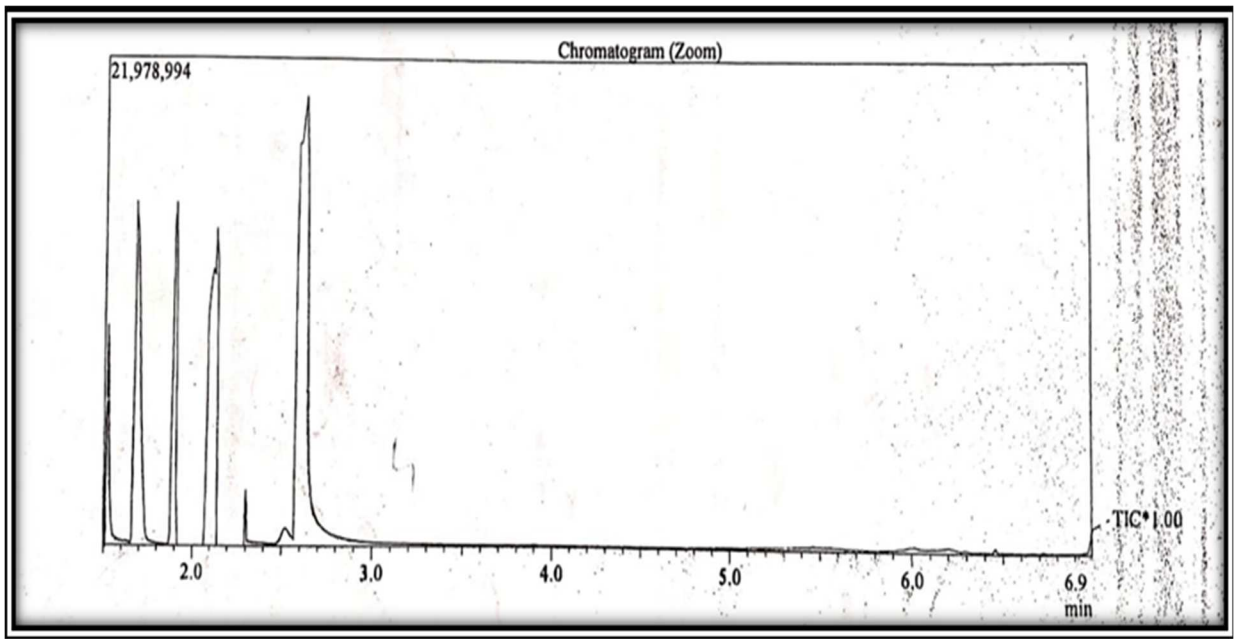


Fig. 4. GCMS spectrum of bioactive compounds identified from leaf extract of *S. lycopersicum* L. (control tomato plants).

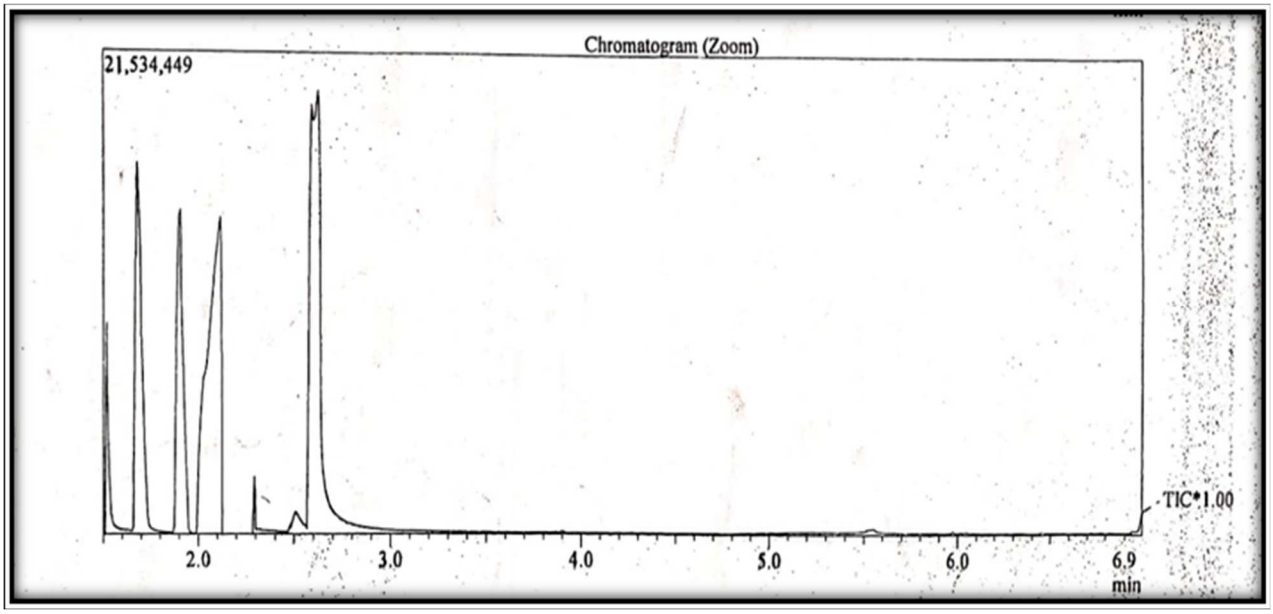


Fig. 5. GCMS spectrum of bioactive compounds identified from the leaf extract of experimental *S. lycopersicum* L. (mastrevirus inoculated tomato plants).

Determination of antimicrobial (antibacterial and antifungal) activity of foliar extracts of control and experimental tomato plants: Plants are great source of highly potent and powerful antimicrobial drugs. An antimicrobial drug is a compound or a substance that kills or slows down the growth of microbes. Quick and effective management of microbial disease is generally achieved by the use of synthetic antibiotics (Marasini *et al*., 2015). The application of synthetic antibiotics can develop the appearance of resistant bacterial strains and cause health hazards in human body (Manandhar *et al*., 2019). Therefore great attention is dedicated to produce new antibacterial agents that are used in the control of resistant pathogenic microorganisms that can curtail the growth of resistant pathogenic bacteria without serious health concerns (Giamarellou, 2010).

Plant extracts have great antimicrobial potential especially against the treatment of infectious diseases caused by resistance microorganisms (Jigna *et al*., 2005). Present study was conducted by considering the antimicrobial potential of control and experimental plant extracts prepared in three different non-polar and polar solvents i.e., chloroform, methanol and distilled water. Among them, methanol is considered as most effective antibacterial solvents for *P. aeruginosa* and *X. campestris*. Against *P. aeruginosa*, control and experimental plants showed maximum resistance i.e., 19 ± 0.57^{a} in methanol. Although in case of resistance against *X. campestris,* control plants showed the maximum value i.e., 27 ± 2.08^{a} and experimental plants showed 21 ± 1.73^{ab} value in methanolic extract (Fig. 6). The similar study was also done by (Koduru *et al*., 2006) who reported that methanolic extract was the most effective polar solvent to extract the *S. aculeastrum* antibacterial agents. These considerations recommended that edible tomato cultivars contained antimicrobial agents which may have various health related benefits (sajet AL-Oqaili and Salman, 2014).

In the present study, chloroform extract of (*S. lycopersicum*) control plants significantly inhibited the *P. verrucosum* growth (30 ± 1.53^{bc}) by comparing the extract in distilled water (26 ± 1.73^{bc}). While in contrary, experimental plants extract in distilled water showed maximum inhibitory effect i.e., 35 ± 3.6^{a} and minimum inhibitory effect 21 ± 1^{c} in chloroform extract against fungi *P. verrucuosum.* Moreover, the chloroform and methanol extracts of control and experimental plants showed noteworthy resistance against *A. flavus* by showing 31 ± 2.65^{a} and 25 ± 2.52^{abc} values respectively (Fig. 6). So, methanol extracts were the most active to inhibit the growth of *A. niger* (Ahmadizadeh *et al*., 2018). The susceptibility of *A. flavus* to the chloroform extracts of experimental plants was notable, as fungus had been implicated in immune deficient patients who often developed opportunistic and superficial candidiasis (Ngane *et al*., 2000; Silva *et al*., 2001). The conclusion of present study was in line with findings of Portillo *et al*. (2001) who reported that *A. niger* was resistant to aqueous, methanol and dichloromethane extracts of fourteen medicinal plants.

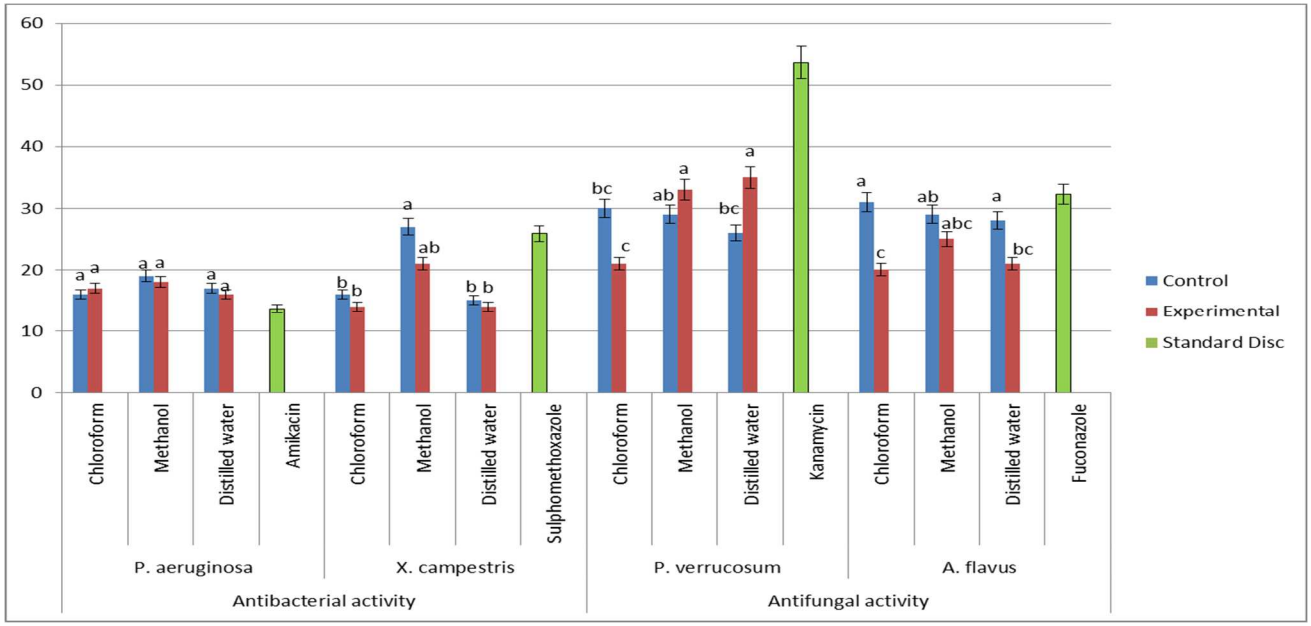


Fig. 6. Graphical-illustration of 'zone-of-inhibition' (mm) created by control and experimental *S. lycopersicum* leaf extracts in various solvents (non-polar *viz*., chloroform and-polar i.e.,/methanol and distilled water) against two pathogenic bacterial strains and two fungal stains i.e., *Pseudomonas aeruginosa*, *Xanthomonas campestris* and *Penicillium verrucosum*, *Aspergillus flavus* respectively.

Antimicrobial activity of various plant species has called attention around the globe; there were various studies that documented strong antimicrobial activity. Such healing properties of plants reduce the occurrence of numerous phytochemicals. Surely the presented findings will a benchmark to search novel compounds from plant extracts prepared in nonpolar and polar solvents (Blot *et al*., 2005; Singh *et al*., 2012).

Determination of antioxidant activity of foliar extracts: In the presented work, control and experimental plant foliar extracts in both non-polar and polar solvents have been employed to analyze the percentage (%) DPPH free radical scavenging activity in three different dilutions *viz*., 1 mg/mL, 0.5 mg/mL and 0.25 mg/mL and results were compared with the available standard antioxidant i.e., ascorbic acid. In this experiment, absorption has been recorded at 695 and 517 nm. Control and experimental leaves of tomato exhibited the uppermost antioxidant value i.e., 4.46 and 5.27 in 0.25 mg/mL in chloroform extract as shown in Fig. 7. These values were close to standard values. Although, distilled water extract in 1 mg/mL dilution showed 0.20 and 1.01 values of control and experimental plants respectively and this was the minimum value in overall experiment. The results of the presented work that tomato asa very rich source of antioxidants werein line with the conclusions of Kevers *et al*. (2007) and Ninfali *et al*. (2005). In present study, the experimental plants infected by mastrevirus had more antioxidants than healthy ones, because when the plants became compromised with any disease, their defense system released more bioactive compounds in order to cope with the disease. Antioxidant profile of tomato fruit could raise with the implementation of the plant defense inducers, which could be two way beneficial i.e. by protecting both plant health and consumer's health (Rendina *et al*., 2019). Earlier, combined consideration of antiviral agents with antioxidants had been deployed for the treatment of influenza related complications (Uchide and Toyoda, 2011).

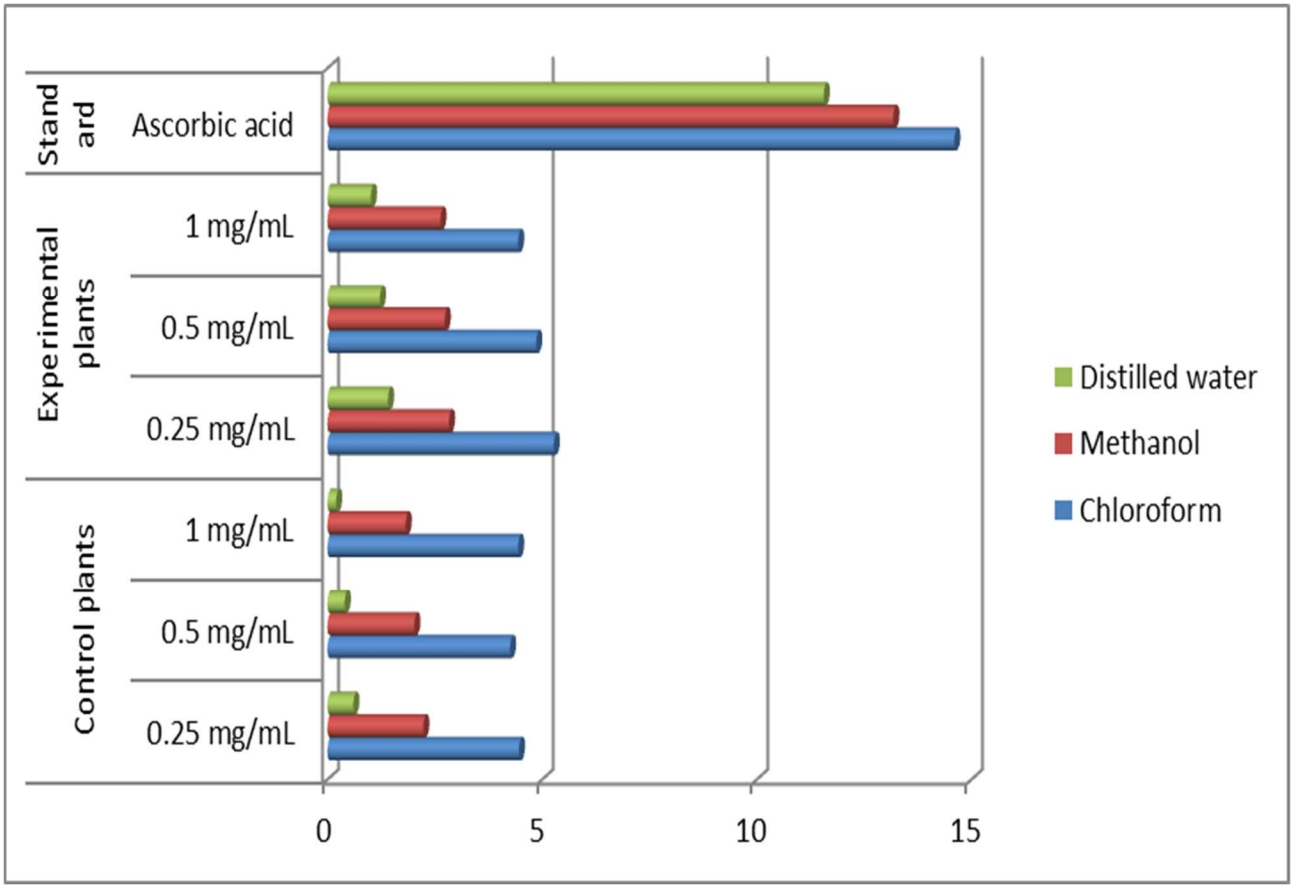


Fig. 7. Graphical illustration of antioxidant activity of control and experimental *S. lycopersicum* leaf extracts non-polar and polar solvents of three different dilutions i.e., 0.25 mg/mL, 0.5 mg/mL and 1 mg/mL by using % DPPH free radical scavenging activity (Absorption at 517 nm).

Table 1: Illustration of bioactive compounds identified through GCMS from leaf extract of *S. lycopersicum* L. (control tomato plants). Structures identified from NIST library.

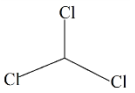
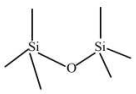
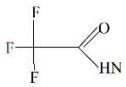
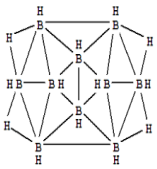
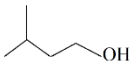
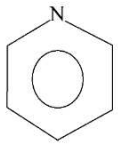
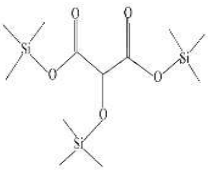
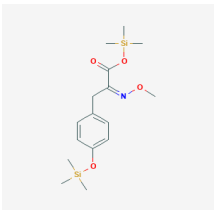
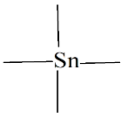
Sr. No.	Compound name	Mol. Formula	Rt. time	Mol. wt. g mol^{-1}	Structure	% Peak area
1.	Trichloromethane	$CHCl_3$	1.675	118		23.14
2.	Disiloxane, hexamethy-	$C_6H_{18}OSi_2$	1.892	162		17.59
3.	Acetamide,2,2,2-trifluoro-N-methyl-	$C_3H_4F_3NO$	2.067	127		19.44
4.	Decaborane	$B_{10}H_{14}$	2.258	124		3.24
5.	1-Butanol, 3-methyl	$C_5H_{12}O$	2.468	88		0.925
6.	Pyridine	C_5H_5N	2.575	79		0.46
7.	Propanedioic acid, [(trimethylsilyl)oxy]-bis (trimethylsilyl) ester	$C_{12}H_{28}O_5SI_3$	5.300	336		26.85
8.	Benzenepropanoic acid, alpha.-(methoxyimino)-4-[(trimethylsilyl)oxy]-, trimethylsilyl ester	$C_{15}H_{26}NO_4Si_2$	5.675	310		2.31

Table 2: Bioactive compounds identified through GCMS from the leaf extract of experimental *S. lycopersicum* L. (mastrevirus inoculated tomato plants). Structures identified from NIST library.

Sr. No	Compound name	Mol. Formula	Rt. time	Mol. wt. g mol^{-1}	Structure	% Peak area
1.	Stannane, tetramethyl-	$C_4H_{12}Sn$	1.700	180		0.5

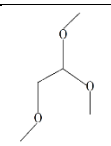
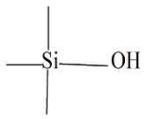
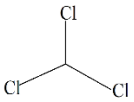
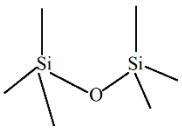
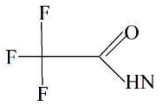
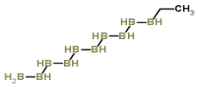
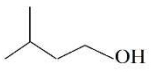
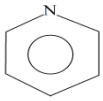
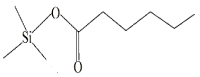
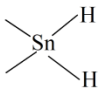
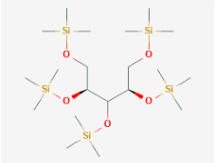
2.	Ethane 1,1,2-trimethoxy-	$C_5H_{12}O_3$	1.517	120		4.4
3.	Silanol, trimethyl-	$C_3H_{10}O$ Si	1.517	90		8.27
4.	trichloromathane	$CHCl_3$	1.675	118		18.04
5.	Disiloxane, haxamethyl-	$C_6H_{18}OSi_2$	1.892	162		13.90
6.	Acetamide,2,2,2-triflouro-N-methyl-	$C_3H_4F_3NO$	2.075	127		15.41
7.	Decaborane, ethyl-	$C_2H_5B_{10}$	2.258	137		18.86
8.	1-Butanol, 3-methyl	$C_5H_{12}O$	2.483	88		2.63
9	Pyridine	C_5H_5N	2.583	79		1.127
10	Octanoice acid, trimethylsilyl ester	$C_{11}H_{24}O_2Si$	6.017	216		0.37
11.	Octadecanal	$C_{18}H_{36}O$	6.825	268		21.42
12.	Stannane, Dimethyl-	C_2H_8Sn	2.275	150		2.82
13.	Hexadecanoic acid, trimethylsilyl ester	$C_{19}H_{40}O_2Si$	6.583	328		19.08
14.	Ribitol,1,2,3,4,5-pentakis-O-(trimethylsilyl)	$C_{20}H_{52}O_5Si_5$	6.992	512		1.41

Table 3: Illustration of same and different bioactive compounds identified in control and experimental *S. lycopersicum* leaf extract.

Sr. No.	Compound name	Control plants	Experimental plants
1.	Ethane 1,1,2-trimethoxy-	-	+
2.	Stannane, tetramethyl-	-	+
	Stannane, dimethyl-	-	+
3.	Disiloxane, hexamethyl-	+	+
4.	Trichloromethane	+	-
5.	Acetamide,2,2,2-trifluoro-N-methyl-	+	+
6.	Decaborane	+	+
7.	1-Butanol, 3-methyl	+	+
8.	Pyridine	+	+
9.	Propanedioic acid, [(trimethylsilyl)oxy]-bis (trimethylsilyl) ester	+	-
10.	Benzenepropanoic acid, alpha.-(methoxyimino)-4-[(trimethylsilyl)oxy]-, trimethylsilyl ester	+	-
11.	Decaborane ethyl-	-	+
12.	Silanol, trimethyl-	-	+
13.	Octanoice acid, trimethylsilyl ester	-	+
14.	Octadecanal	-	+
15.	Hexadecanoic acid, trimethylsilyl ester	-	+
16.	Ribitol,1,2,3,4,5-pentakis-O-(trimethylsilyl)	-	+

Table 4. Illustrates presence/absence of phytochemicals in control and experimental plant leaves extracts of *Solanum lycopersicum.* Presence or absence of compounds is indicated by '+' or '-' sign respectively.

Sr. no.	Test name	Plant parts	Result	Inference
1.	Alkaloids	Control plants	Creamish precipitate/orange precipitate	++
		Experimental plants		++
2.	Saponins	Control plants	persistent froth	+
		Experimental plants		+
3.	Coumarins	Control plants	No yellow florescence detected	-
		Experimental plants		-
4.	Terpenoids	Control plants	blue-green ring appeared	+
		Experimental plants		++
5.	Tannins	Control plants	brownish- green coloration appeared	++
		Experimental plants		+
6.	Phlobatannins	Control plants	red precipitation	++
		Experimental plants		+
7.	Cardiac glycoside	Control plants	blue green coloration	++
		Experimental plants		+
8.	Anthraquinones	Control plants	reddish pink color appeared	++
		Experimental plants		+
9.	Flavonoids	Control plants	Yellow/reddish orange color appeared	++
		Experimental plants		+
10.	Phenolic compounds	Control plants	Green color appeared	+
		Experimental plants		++

Note: ++ Strongly present + Moderately present negative

Conclusion: In conclusion, comparative bio accessibility of the metabolites, phytochemicals, antimicrobial and antioxidants of mastrevirus infected and healthy tomato plants were determined in this study. Experimental plants were proved to be having increase amount of phytochemicals, bioactive compounds and antioxidants than control healthy plants. Hence fourteen and eight metabolites have been identified in experimental and control plants respectively. Moreover, both experimental and control plants showed strong antimicrobial potential in different solvents. Methanolic extract was substantiated solvent against bacteria whilst chloroform and distilled water leaves extracts were used as strong antifungal solvents.

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REFERENCES

Ahmadizadeh, C., A. Monadi., A. Rezaie., M.G. Rad and B. Jafari (2018). Antibacterial activity of methanolic extract and essence of Sagebrush (*Artemisia vulgaris*) against pathogenic bacteria. Life Sci. J. 15(5).

Ajaib, M., S. Ali and Z. Khan (2014). Antioxidant and antimicrobial activities of an ethnobotanically important plant *Notholirion thomsonianum* from district Kotli, Azad Jammu & Kashmir. J. Anim. Plant Sci., 24(3): 774-780.

Beecher, G.R (2003). Overview of dietary flavonoids: nomenclature, occurrence and intake. J. Nutr. 133(10): 3248-3254. DOI: 10.1093/jn/133.10.3248S

Bellens, K., P. Mészáros., S. Vermeir., D. Kirsanow., A. Legin., S. Buysens., N. Cap., B.M. Nicolaï., L. Lammertyn (2008). Analysis of tomato taste using two types of electronic tongues. Sens. Actuator. B. Chem. 131: 10–17. DOI:10.1016/j.snb.2007.12.024

Blot, S., D.D. Bacquer., E. Hoste., P. Depuydt., K. Vandewoude., J.D. Waele., D. Benoit., J. Schuijmer., F. Colardyn and D. Vogelaers (2005). Influence of matching for exposure time on estimates of attributable mortality caused by nosocomial bacteremia in critically ill patients. Infect. Control Hosp. Epidemiol. 26(4): 352-356. DOI: https://doi.org/10.1086/502551

Doyle, J (1991). DNA protocols for plants. In Molecular techniques in taxonomy. Springer Berlin Heidelberg. 283-293. https://doi.org/10.1007/978-3-642-83962-7_18

Erasto, P., Bojase-Moleta, G and Majinda, R.R (2004). Antimicrobial and antioxidant flavonoids from the root wood of *Bolusanthus speciosus*. Phytochemistry. 65(7): 875-880. https://doi.org/10.1016/j.phytochem.2004.02.011

Ferreira, M.J.U., A. Duarte and J.R. Ascenso (1996). Antimicrobial activity and phytochemical study of *Euphorbia tuckeyana*. Fitoterapia. 67(1): 85-86. doi: 10.4103/2229-516X.117082

Fiallo-Olivé, E., J.M. Lett., D.P. Martin., P. Roumagnac., A. Varsani., F.M. Zerbini., J. Navas-Castillo and I.R. Consortium (2021). ICTV virus taxonomy profile: Geminiviridae 2021. J. G. Virol. 102(12): 001696. DOI: 10.1099/jgv.0.001696

Fiehn, O and Kind, T (2007). Metabolite profiling in blood plasma. In Metabolomics. 3-17. Humana Press. DOI: 10.1007/978-1-59745-244-1_1

Filannino, P., G. Cardinali., C.G. Rizzello., S. Buchin., M.D. Angelis., M. Gobbetti and R.D. Cagno (2014). Metabolic responses of *Lactobacillus plantarum* strains during fermentation and storage of vegetable and fruit juices. Appl. Environ. Microbiol. 80: 2206-2215. DOI: 10.1128/AEM.03885-13

Fuke, S and Shimizu, T (1993). Sensory and preference aspects of umami. Trends. Food. Sci. Technol. 4: 246–251. https://doi.org/10.1016/0924-2244(93)90139-2

Giamarellou, H (2010). Multidrug-resistant Gram-negative bacteria: how to treat and for how long. Int. J. Antimicrob. Agents. 36: 50-54. DOI: 10.1016/j.ijantimicag.2010.11.014

Gullberg, R.C., J.J. Steel., S.L. Moon., E. Soltani and B.J. Geiss (2015). Oxidative stress influences positive strand RNA virus genome synthesis and capping. Virology. 475: 219-229. doi: 10.1016/j.virol.2014.10.037

Hanssen, I.M., I. Gutiérrez-Aguirre., A. Paeleman., K. Goen., L. Wittemans., B. Lievens., A.C.R.C. Vanachter., M. Ravnikar., B.P.H.J. Thomma (2010b). Cross-protection or enhanced symptom display in greenhouse tomato co-infected with different *Pepino mosaic virus* isolates. Plant. Pathol. 59: 13-21. https://doi.org/10.1111/j.1365-3059.2009.02190.x

Hanssen, I.M., M. Lapidot and B.P. Thomma (2010a). Emerging viral diseases of tomato crops. Mol. Plant Microbe Interact. 23(5): 539-548. DOI: 10.1094/MPMI-23-5-0539

Jamil, M., B. Mirza., A. Yasmeen and M.A. Khan (2012). Pharmacological activities of selected plant species and their phytochemical analysis. J. Med. Plant Res. 6 (37): 5013-5022. DOI:10.5897/JMPR09.259

Janningsmeier, S.D and McFeeters, R.F (2015). Metabolic footprinting of *Lactobacillus buchneri* strain LA1147 during anaerobic spoilage of fermented cucumbers. Int J Food Microbiol. 215: 40–48. https://doi.org/10.1016/j.ijfoodmicro.2015.08.004

Jigna, P., N. Rathish and C. Sumitra (2005). Preliminary screening of some folklore medicinal plants from western India for potential antimicrobial activity. Indian J Pharmacol. 37: 408-409.

Karacabey. E and G. Mazza (2010). Optimisation of antioxidant activity of grape cane extracts using response surface methodology. Food Chem. 119(1): 343-348. https://doi.org/10.1016/j.foodchem.2009.06.029

Kazimierczak, R., E. Hallmann., J. Lipowski., N. Drela., A. Kowalik., T. Püssa., D. Matt., A. Luik., D.

Gozdowski and E. Rembialkowska (2004). Beetroot (*Beta vulgaris* L.) and naturally fermented beetroot juices from organic and conventional production: metabolomics, antioxidant levels and anticancer activity. J. Sci. Food. Agric. 94: 2618-2629. DOI:10.1002/jsfa.6722

Kevers, C., M. Falkowski., J. Tabart., J.O. Defraigne., J. Dommes and J. Pincemail (2007). Evolution of antioxidant capacity during storage of selected fruits and vegetables. J. Agric. Food Chem. 55(21): 8596-8603. DOI: 10.1021/jf071736j

Khalid, S., Shamim, F., Bibi, S., Javaid, S and Khan, F (2016). Antimicrobial potential of female cones and needles of chir pine (*Pinus roxburghii* Sargent). Pakistan J. Phytopathol. 28(2): 193-199.

Khokhar, K.M and HRI, N (2013). Present status and prospects of tomatoes in Pakistan. *Agricultural Corner-Farmers to Global Market*. 1-21. DOI:10.13140/RG.2.2.15944.57605

Kim, J.S., Z. Chen., T.L. Alderete., C. Toledo-Corral., F. Lurmann., K. Berhane and F.D. Gilliland (2019). Associations of air pollution, obesity and cardiometabolic health in young adults: The Meta-AIR study. Environ. Int. 133: 105180. doi: 10.1016/j.envint.2019.105180

Koduru, S., D.S. Grierson and A.J. Afolayan (2006). Antimicrobial Activity of *Solanum aculeastrum*. Pharm boil. 44(4): 283-286. DOI:10.1080/13880200600714145

Koek, M.M., B. Muilwijk., M.J.V.D. Werf and T. Hankemeier T (2006). Microbial metabolomics with gas chromatography/mass spectrometry. Anal Chem. 78: 1272-1281. https://doi.org/10.1021/ac051683+

Kumar, S., U.K. Misra., J. Kalita., V.K. Khanna and M.Y. Khan (2009). Imbalance in oxidant/antioxidant system in different brain regions of rat after the infection of Japanese encephalitis virus. Neurochem. Int. 55: 648-654. DOI: 10.1016/j.neuint.2009.06.008

Manandhar, S., S. Luitel and R.K. Dahal (2019). *In Vitro* Antimicrobial activity of some medicinal plants against human pathogenic bacteria. J. Trop. Med. 19: 1-5. DOI: 10.1155/2019/1895340

Marasini, B.P., P. Baral., P. Aryal., K.R. Ghimire., S. Neupane., N. Dahal., A. Singh., L. Ghimire and K. Shrestha (2015). Evaluation of antibacterial activity of some traditionally used medicinal plants against human pathogenic bacteria. BioMed Res. Int. 15: 1-6. https://doi.org/10.1155/2015/265425

Ngane, N.A., L. Biyiti., P.H.A. Zollo and P.H. Bouchet (2000). Evaluation of antifungal activity of extracts of two *Cameroonian Rutaceae*: *Zanthoxylum leprieurii*. Gruill et Perr. and *Zanthoxylum xanthoxyloides*. Waterm. J Ethnopharmacol. 76: 347–354. DOI:10.1016/S0378-8741(99)00188-9

Nice, K (2013). Antimicrobial screening of secondary metabolites from Solanaceae (Doctoral dissertation, Royal Holloway, University of London).

Ninfali, P., G. Mea., S. Giorgini., M. Rocchi and M. Bacchiocca (2005). Antioxidant capacity of vegetables, spices and dressings relevant to nutrition. Br. J. Nutr. 93(2): 257–266. DOI: https://doi.org/10.1079/BJN20041327

Portillo, A., R. Vila., B. Freixa., T. Adzet and S. Canigueral (2001). Antifungal activity of Paraguayan plants used in traditional medicine. J Ethnopharmacol. 76: 93–98. DOI: 10.1016/s0378-8741(01)00214-8

Rendina, N., M. Nuzzaci., A. Sofo., P. Campiglia., A. Scopa., E. Sommella., G. Pepe., M. De Nisco., M.G. Basilicata and M. Manfra (2019). Yield parameters and antioxidant compounds of tomato fruit: the role of plant defence inducers with or without Cucumber mosaic virus infection. J Sci. Food Agric. 99(12): 5541-5549. https://doi.org/10.1002/jsfa.9818

Rossouw, L.T., N.E. Madala., F. Tugizimana., P.A. Steenkamp., L.L. Esterhuize and I.A. Dubery (2019). Deciphering the resistance mechanism of tomato plants against whitefly-mediated tomato curly stunt virus infection through ultra-high-performance liquid chromatography coupled to mass spectrometry (UHPLC-MS)-based metabolomics approaches. Metabolites. 9(4): 60. DOI: 10.3390/metabo9040060

sajet AL-Oqaili, R.M and B.B.M.M.A. Salman (2014). *In Vitro* Antibacterial Activity of *Solanum Lycopersicum* Extract against some Pathogenic Bacteria. *In Vitro*. 27: 12-18.

Santi, L., L. Batchelor., B. Hjelm., J. Kilbourne., C.J. Arntzen., Q. Chen and H.S. Mason (2008). An efficient plant viral expression system generating orally immunogenic Norwalk virus-like particles. Vaccine. 26(15): 1846-1854. DOI: 10.1016/j.vaccine.2008.01.053

Schreck, R., P. Rieber and P.A. Baeuerle (1991). Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-kappa B transcription factor and HIV-1. EMBO J. 10: 2247-2258. DOI: 10.1002/j.1460-2075.1991.tb07761.x

Silva, M.V., T.R. Costa., M.R. Costa., E.C. Ferrera., O.F.L. Fernandes., S.C. Santos., L.M. Liao., P.H. Ferri., J.R. Paula., H.D. Ferriera and M.R.R. Silva (2001). Growth inhibition effect of Brazilian Cerrado plant extracts on Candida

species. Pharm Biol. 39: 138-141. https://doi.org/10.1076/phbi.39.2.138.6248

Singh, A.G., A. Kumar and D.D. Tewari (2012). An ethnobotanical survey of medicinal plants used in Terai forest of western Nepal. J Ethnobiol Ethnomed. 8(1): 19. https://doi.org/10.1186/1746-4269-8-19

Steel, R.G.D and J.H. Torrie (1960). Principles and Procedures of Statistics. New York, McGraw-Hill.

Terao, J., Y. Minami., N. Bando (2010). Singlet molecular oxygen-quenching activity of carotenoids: relevance to protection of the skin from photoaging. J. Clin. Biochem. Nutr. 48(1): 57. DOI: 10.3164/jcbn.11-008FR

Uchide, N and H. Toyoda (2011). Antioxidant therapy as a potential approach to severe influenza associated complications. Molecules. 16: 2032-2052. doi: 10.3390/molecules23100000

Yilmaz, E (2001). The chemistry of fresh tomato flavor. Turk J Agric For. 25: 149-155. https://journals.tubitak.gov.tr/agriculture/vol25/iss3/1

Yuwei, Q.I (2016). Characterization of Phytochemicals and Antioxidant Activities of Specialty Tomatoes. PhD dissertation. http://hdl.handle.net/10214/9663

Zia-Ur-Rehman, M., H.W. Herrmann., M.J. Iqbal., M.S. Haider and J.K. Brown (2015). First report of *Chickpea chlorotic dwarf virus* infecting tomato crops in Pakistan. Plant Disease. 99 (9): 1287-1287. https://doi.org/10.1094/PDIS-11-16-1626-PDN

Zushi, K and N. Matsuzoe (2011). Utilization of correlation network analysis to identify differences in sensory attributes and organoleptic compositions of tomato cultivars grown under salt stress. Sci Hortic. 129: 18-26. DOI:10.1016/j.scienta.2011.02.011.