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PRELIMINARY PHYTOCHEMICAL SCREENING OF HEALTHY *Solanum lycopersicum L.* LEAVES USING GAS CHROMATOGRAPHY- MASS SPECTROMETRY (GC-MS)

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ABSTRACT

Solanum lycopersicum (Tomato) is one of the most consumed fruit vegetables and one of the best sources of phytochemicals which makes a significant contribution to the daily intake of rich bioactive compounds. This study was conducted to identify the phytochemical characteristics of healthy *S. lycopersicum* leaves, mostly regarded as a waste, using Gas chromatography-Mass spectrometry (GC-MS) technique to determine the chemical constituents. Fresh matured leaves of *S. lycopersicum* were washed with sterile distilled water to remove contaminants. They were air dried and pulverized into semi-coarse powder which was later extracted using 70% methanol. The preliminary phytochemical analysis was carried out using standard procedures. Another sample of the leaves was dried and pulverized to powder in a mechanical grinder, weighed and transferred to a flask. It was treated with petroleum ether, incubated overnight and strained through a Whatman filter paper. The filtrate was then concentrated and analyzed using Gas chromatography-Mass spectrometry (GC-MS). Qualitative phytochemical analysis showed absence of anthraquinones glycosides, cardiac glycosides, tannins, and terpenoids, while saponins, phenols, phlobatannins, alkaloids, steroids, and flavonoids were present. The GC-MS identified fourteen compounds in the petroleum ether extract of *Solanum lycopersicum* leaves which include: Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-; γ-Terpinene; Terpinen-4-

ol; Bicyclo[3.1.0]hex-3-en-2-one, 4-methyl-1-(1-methylethyl)-; Eugenol; Humulene; Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-; Caryophyllene oxide; 1H-3a,7-Methanoazulene, octahydro-1,4,9,9-tetramethyl-; Benzene, 1-(1,1-dimethylethyl)-3-methyl; Thymol; Phenol, 2-ethyl-4,5-dimethyl-; Dimethyl phthalate and Pyrazine, 2,3-diethyl-5-methyl-.*Solanum lycopersicum* leave extract contains phyto-constituents that may have potentials for pharmacological activities.

Keywords: *Solanum lycopersicum*, medicinal plant, phytochemical screening, GC-MS

INTRODUCTION

Almost 80% of the world's population use medicinal plants or their metabolites for primary health care or treatment (Ege *et al*., 2021; Belani *et al*., 2022). Quite a number of secondary metabolites such as phenol, tannins, saponins, alkaloids and flavonoids have been discovered in several medicinal plant species. They are extensively used in treating various infections and conditions, like cancer, diabetes, inflammation, neurodegenerative diseases and also in mortal remedy, husbandry etc. Plants have played a huge part in medicine for a long time and these are deduced from the leaves, bark, fruits, seeds, roots and flowers (Craig *et al*., 2001). These phytoconstituents are highly abundant in *S. lycopersicum*. Solanaceae family comprises of 98 genera and 2,700 species with an extensive range in morphology, ecology, along with habitat inclusiveness. Due to the resemblance of the flowers to the brightness of the sun and its radiation, some *Solanum* species are known as "sun berries." A large percentage of the species are classified as perennials or annual climbers (Griffin & Lin, 2000; Thulasi *et al*., 2020).

The fruit is one of the most consumed fruit vegetables and one of the best sources of antioxidants, which makes a significant contribution to the daily intake of rich bioactive compounds. It is eaten either fresh or processed, such as in stews, soups, canned tomato, sauce, and juice (Rajan *et al*., 2022).
Etiological exploration findings had formerly set up a link among both eating raw tomatoes and foods containing tomato constituents and have reduced chance of getting cancer and cerebrovascular problems (Rodriguez-Amaya *et al*., 2019; Storniolo *et al*., 2020). Vitamins like ascorbic acid, tocopherols, and phenolic composites like flavonoids and hydroxycinnamic acid derivatives are among the tomato-based anti-inflammatory compounds known as pigments that are similar to carotene, an early form of vitamin A pigment, and vitamin C (López-Yerena *et al*., 2024). Through free-radical scavenging, essence chelation, inhibition of cellular proliferation, and modulation of enzymatic exertion and signal transduction processes, such composites may play an essential function in inhibiting reactive oxygen compounds, known to be responsible for a number of serious conditions (Crozier *et al*., 2009; Saha *et al*., 2019).

There are approximately 300,000 plant species that have phytoconstituents found in plants (Březinová *et al.* 2018 and Mishra *et al*., 2021), which have been studied by identifying a wide range of structural variations and each one has unique properties that make it useful for a variety of tasks. It is critical for good nutrition to consume a number of plants which are rich in nutrients such as vitamins and minerals.

Plants produce numerous other metabolites, which are also significant. The roles and modes of action of these metabolites vary. A large source of knowledge for a variety of chemical elements that could be created as medications with exquisite selectivity is found in medicinal plants with addition to its therapeutic uses. These variables have the sources of potentially advantageous medicinal products that ought to offer novel insights as well as pointers in growth and development of contemporary medications (Gajalakshmi *et al.,* 2012; Srivastava *et al.*, 2021). Alkaloids, tannins, flavonoids, and phenolic compounds rank as the top four bioactive components of plants (Rabecca *et al.*, 2022).

Plants containing phenolics and flavonoid compounds are an important family of biomolecules with numerous therapeutic properties for humans. The basic structure of phenolic compounds like gallic acid, caffeic acid, ferulic acid, protocatechuic acid and coumaric acid is composed of a phenolic (C_6H_5OH) ring, carboxylic acid (COOH) and hydroxyl groups (OH). Polyphenolic compounds known as flavonoids are further classified into sub-classes including flavanols, flavanones, flavones, flavanonols, flavan-3-o1s and iso-flavones (Koleva *et al.*, 2021). Flavonoids and phenolic compounds have a direct relationship with each other's antioxidant properties due to the presence of hydroxyl (OH) groups in these compounds which also control their ability to scavenge free radicals. The pharmacological effects of phenolic compounds include antibacterial, phytonutrients, and anticancer properties (Sun *et al.,* 2019).

Understanding the relationship between phytoconstituents and plant bioactivity is important for the synthesis of molecules with targeted therapeutic properties for a variety of illness, including chronic diseases (Konappa *et al.*, 2020). Previous studies on *Solanum lycopersicum* fruit have revealed the presence of metabolites which has validated its antioxidant, anticancer, cytotoxic, antiviral and antimicrobial potentials (Nityasree *et al.*, 2020; Mallick, 2021; Mohammed *et al.*, 2022). Agricultural industry by-products and wastes form a significant proportion of worldwide agricultural productivity (Figueiredo-Gonzalez *et al.*, 2016). In the recent time, the trend in the agribusiness sector is to recover, to evaluate, and to find better uses for all their by-products, such as, peels, seeds, stems, and leaves. Tomato is the third most important vegetable grown in the world (Coelho *et al.*, 2019). Despite dart information available concerning non-edible plant organs, they constitute valuable by-products for the food, cosmetic and pharmaceutical industry (Chukwuma, 2024). Along with the level of cultivation for fruits, the leaves can also be useful for some other purposes.

This study investigated the phytochemical constituents of healthy *Solanum lycopersicum* leaves, exploring their potential applications in the pharmaceutical industry and providing a valuable opportunity for farmers to convert waste into a source of income.

METHODS

Plant collection

Solanum lycopersicum (heirloom) with determinate growth habit was grown without fertilizer in a Departmental farm within Olabisi Onabanjo University campus Ago-Iwoye, Ogun State, Nigeria. Matured leaves were later collected and Identified at the Department of Plant Science. Herbarium specimen was also deposited at El-Kafar herbarium of same Department with herbarium number-EH/2015/14006.

Plant preparation

Solanum lycopersicum leaves were properly washed with running water from the faucet and once with sterile distilled water to remove any surface dust and contaminants before being air dried at room temperature on a sterile blotter for 3 days. Air drying served to protect the plant's active components from UV radiation and deterioration. Using a laboratory mortar and pestle, the dried leaves were ground into a coarse powder after being completely dried. The plant material which has been ground into a semi-powder was weighed before storage in an airtight container and maintained in a dimly lit area for the extraction process.

Plant Extraction

Solanum lycopersicum leaves (133 g) was macerated in 160 ml of methanol in a tightly-sealed Pyrex glass container for 3 days. The jar was shaken rigorously daily; the closed jar was kept at room temperature in the laboratory. The phytoconstituents were obtained using a laboratory glass funnel that contained modified cotton wool, and later by using filter paper. The plant extract was concentrated by evaporation of the solvent, using water bath at controlled temperature.

Phytochemical screening

Preliminary phytochemical assay was carried out to detect the presence of alkaloids, anthraquinones glycosides, cardiac glycosides, saponins and flavonoids in the leaf methanol extract using the recommended methods of Sofowora (2008) and Chukwuemeka (2020).

Test for Alkaloids

After filteration, the extract was allowed to cool; the pH was reduced to about 6.0 -7.0 by using litmus paper and 10 ml of Wagners and Dragendorff's reagents were added in very small amounts to 5 ml of the filtrate in separate test tubes (Sofowora, 2008; Zohra *et al.,* 2012)

Test for Anthraquinone glycoside (The Borntrager's test)

The plant extract was boiled for a period of five minutes, filtered while it was still hot, and allowed to cool. It was tested with 2 ml of 10% Hydrochloric acid (HCl). An equal volume (aliquot) of chloroform was divided among the filtrates solutions and gently shaken. 10% ammonia solution was poured into the test tube after the lower layer of chloroform had been transferred there, and the tube was gently shaken. A thin layer of rose-pink colour on the solution served as a sign that anthraquinone glycosides were present (Sofowora, 2008).

Test for Cardiac glycoside

Keller-Kiliani Test: Examining the leaf extract, 2 ml of acetic acid and water were combined with one drop of the Ferric chloride reagent; 1ml of concentrated Tetraoxosulphate (VI) acid was cautiously added and the formation of a reddish-brown ring at the interface of the liquid was an indicator of the presence of cardiac glycoside.

Test for Flavonoids

One gramme (1.0 g) of the powdered leaves was boiled with 10 ml of water for 5 minutes, filtered while hot and allowed to cool.

The following reagents were added to the filtrate:

Two mls of sodium hydroxide solution. A yellow colouration indicated the presence of flavonoid in the plant sample

Also, 5 ml dilute ammonia solution was added to 5 ml of the aqueous filtrate and conc. H_2SO_4.. The presence of a light brown colour at the bottom and a reddish interface which disappeared on standing suggested the presence of flavonoid in the plant sample.

Test for Tannins (The Braemer's test)

Two millilitres of the plant extract was treated with 10% alcoholic ferric chloride solution and observed for formation of blue or greenish colouration (Willie *et al.*, 2021).

Test for Saponins

Six millilitres of water was added to 2 mls of plant extract in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins (Willie *et al.*, 2021).

Test for Phenols

Ferric chloride solution was added to a mixture of ten milliliters (10 mls) of ethanol and plant extract; the presence of phenol was confirmed by dark colouration (green, blue or purple) -Willie *et al.*, 2021.

Test for Steriods

An aliquot of 2 ml of acetic anhydride was added to 2 ml of plant sample extract which was followed by careful addition of 2 ml H_2SO_4. The colour changed from violet to blue or green indicating the presence of steroids (Sofowora, 2008).

Test for Terpenoids (Salkowski test)

An aliquot containing 5 ml of extract was added to 2 ml of chloroform and 3 ml of concentrated H_2SO_4 cautiously incorporated, a reddish-brown colour change at the interphase indicated the occurrence of terpenoids (Ahamad *et al.*, 2023).

Test for Phlobatannins

Aqueous hydrochloric acid (1 ml) was stirred with the plant extract (2 ml), boiled and allowed to cool down. Availability of phlobatannins was implicated in precipitate (red) observed (Sofowora, 2008).

Preparation of extracts for GC-MS analysis

The sample was dried and pulverized to a powder in a mechanical grinder. Required quantity of the leaf powder of *S. lycopersicum* was weighed and transferred to a flask, treated with petroleum ether until the powder was fully immersed; it was incubated overnight, and strained through a Whatman No.41 filter paper. The filtrate was then concentrated to 1 ml by bubbling nitrogen gas into the solution and 2 µl sample of the solution was employed in GC-MS for analysis of different compounds.

GC-MS analysis of phytochemicals

The combination of Gas chromatography with the identification capabilities of Mass spectrometry results in identification of components of mixtures of compounds including plant extracts.

The resulting data have a tri-dimensional nature, from which retention times (RT), chromatographic areas, and mass spectra can be obtained for every individual component of a complex mixture. Petroleum ether extract of *Solanum lycopersicum* was analyzed by GC-MS method. The GC-MS technique was done by using GC Shimadzu QP2010 system and GC interfaced to a MS. It is equipped with Elite-1 fused silica capillary column. Helium gas (99.99%) was employed as the carrier gas at a constant current rate of 1.51 cc/minute and an injection volume of 2 µl was employed (split ratio 1:20). Injection temperature was 200°C; ion source temperature 200°C. The oven temperature was programmed from 100°C (isothermal for 2 minutes), with an increase of 300°C for 10 minutes. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds with a scan range of 40-1000 m/z. Total GC running time was 35 minutes (Odewo *et al.*, 2023).

Component identification of GC-MS Result

The interpretation of GC-MS spectra was carried out using the National Institute of Standards and Technology (NIST) database, which contains over 62,000 entries. The unknown component's mass spectrum was compared to the spectrum of known components contained in the NIST library. The components of the test materials were identified by name, molecular weight (MW), and structure (Odewo *et al.*, 2023).

RESULTS

Preliminary phytochemical Analysis

The preliminary phytochemical assay of *S. lycopersicum* methanol leaf extract revealed the presence of Alkaloids, Flavonoids, Saponins, Phenols, Steroids and Phyloba-tannins. While Anthraquinone glycoside, Cardiac glycoside, Tannins and Terpenoid were completely absent (Table 1).

Table 1: Preliminary Phytochemical Constituents of S*olanum lycopersicum* leaf extract

Test	Inference
Alkaloids	++
Anthraquinones	-
Cardiac glycosides	-
Flavonoids	+
Tannins	-
Saponins	++
Phenols	++
Steroids	+++
Terpenoids	-
Phlobatannins	++

Key: - (completely absent), + (present in a minute or trace amount), ++ (present in a moderate amount), +++ (present in an appreciable amount).

GC-MS result of *Solanum lycopersicum* leaf extract

Fourteen compounds were identified in the result of the GC-MS analysis of the leave extract of the studied plant. The identified bioactive phytocomponents have been reported to have anti-inflammatory, anti-cancer, antimicrobial activities. (Ahmed *et al.*, 2021; Cuevas-Cianca *et al.*, 2023).

The analysis of GC-MS carried out on the petroleum ether extract from *S. lycopersicum*, revealed a total of 14 phytoconstituents were detected (Table 2). Their molecular weights (MW) ranged from 136.23.to 220.35 while the retention time (RT) ranged from 3.36 to 16.16.

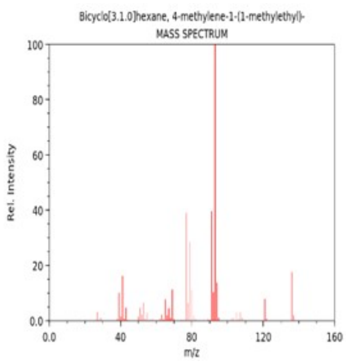
Using the peaks to identify the compounds detected in the leave extract (Figure 1), the result revealed Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)- (sabinene) to have the least RT (3.36) and MW of 150.21 while Pyrazine, 2,3-diethyl-5-methyl- had 16.16 and 136.23. Close to this is Dimethyl phthalate (DMP) with RT and MW of 15.52, 194.18 respectively.

Sabinene, is a natural bicyclic monoterpene with various medicinal properties which include antifungal, antimicrobial, anti-inflammatory, antioxidant and anticancer (Krifa *et al.*,2015; Zheljazkov *et al.*, 2017; Ishak *et al.*, 2024; Judžentienė *et al.*, 2024).

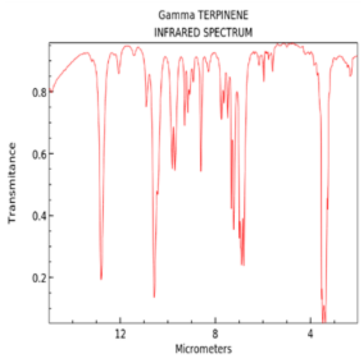
DMP has been used as solvent, insect repellant and plasticizer in various products, such as plastics, resins, and adhesives.

Table 2: Phytoconstituents identified in the leaf extract of *Solanum lycopersicum* by GC-MS

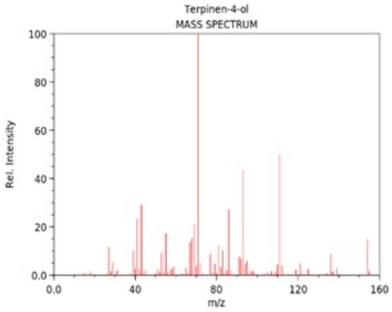
S/N	RT	Name of compound	Molecular formular	Molecular weight
1	3.36	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	$C_{10}H_{14}O$	150.21
2	4.25	γ-Terpinene	$C_{10}H_{16}$	136.23
3	5.67	Terpinen-4-ol	$C_{10}H_{18}O$	150.21
4	6.71	Bicyclo[3.1.0]hex-3-en-2-one, 4-methyl-1-(1-methylethyl)-	$C_{10}H_{14}O$	154.24
5	7.90	Eugenol	$C_{10}H_{12}O_2$	164.20
6	8.90	Humulene	$C_{15}H_{24}$	204.35
7	9.65	Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-	$C_{15}H_{24}$	204.35
8	10.19	Caryophyllene oxide	$C_{15}H_{24}O$	220.35
9	11.43	1H-3a,7-Methanoazulene, octahydro-1,4,9,9-tetramethyl-	$C_{15}H_{26}$	206.36
10	12.91	Benzene, 1-(1,1-dimethylethyl)-3-methyl-	$C_{11}H_{16}$	148.24
11	13.78	Thymol	$C_{10}H_{14}O$	150.21
12	14.09	Phenol, 2-ethyl-4,5-dimethyl-	$C_{10}H_{14}O$	150.21
13	15.52	Dimethyl phthalate	$C_{10}H_{10}O_4$	194.18
14	16.16	Pyrazine, 2,3-diethyl-5-methyl-	$C_{10}H_{16}$	136.23



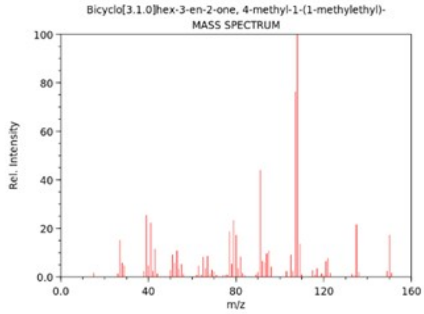
A



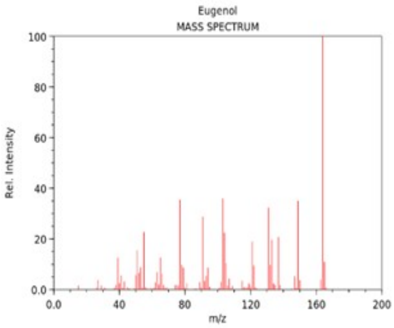
B


Terpinen-4-ol
MASS SPECTRUM
Rel. Intensity
m/z

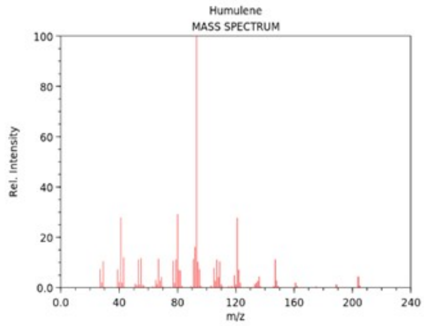
C


Bicyclo[3.1.0]hex-3-en-2-one, 4-methyl-1-(1-methylethyl)-
MASS SPECTRUM
Rel. Intensity
m/z

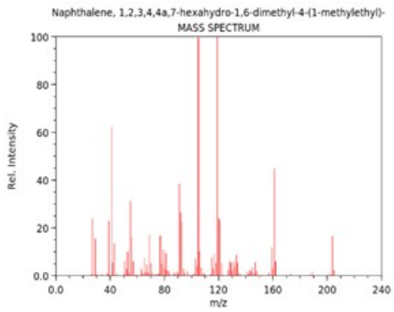
D


Eugenol
MASS SPECTRUM
Rel. Intensity
m/z

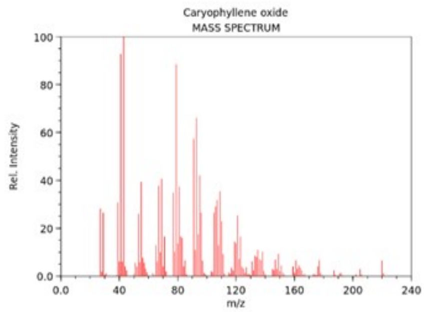
E


Humulene
MASS SPECTRUM
Rel. Intensity
m/z

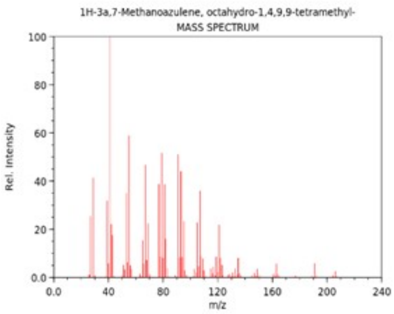
F


Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-
MASS SPECTRUM
Rel. Intensity
m/z

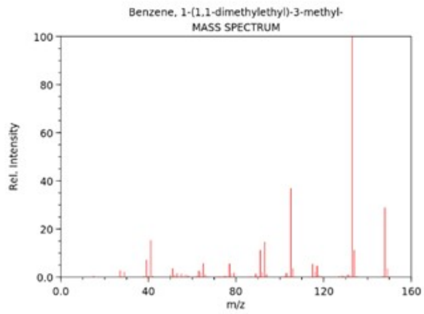
G


Caryophyllene oxide
MASS SPECTRUM
Rel. Intensity
m/z

H


1H-3a,7-Methanoazulene, octahydro-1,4,9,9-tetramethyl-
MASS SPECTRUM
Rel. Intensity
m/z

I


Benzene, 1-(1,1-dimethylethyl)-3-methyl-
MASS SPECTRUM
Rel. Intensity
m/z

J

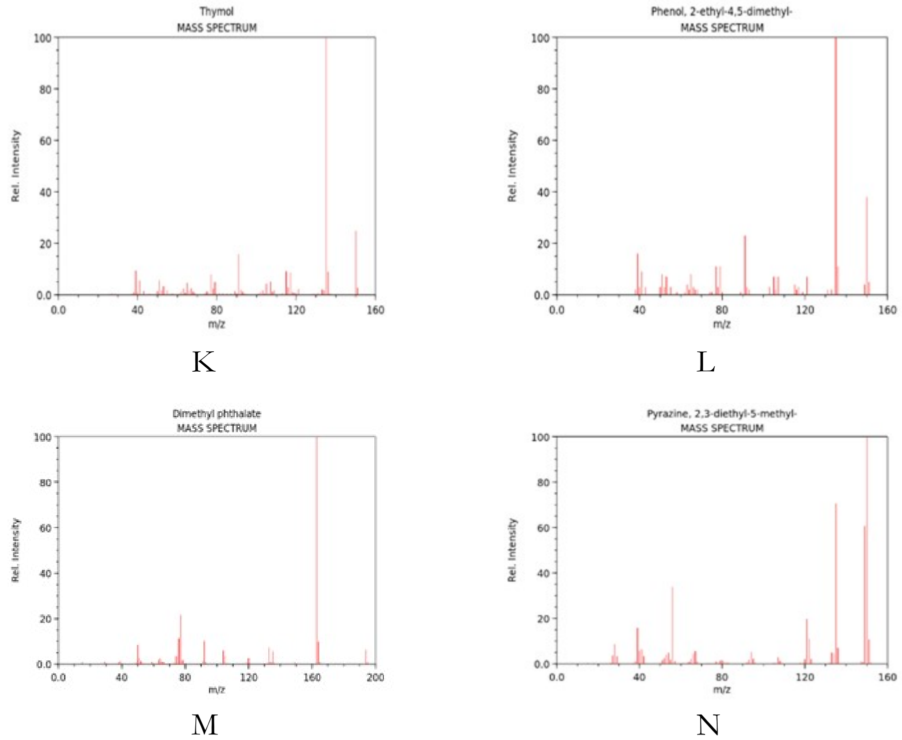


Figure 1: Gas chromatograph-mass spectrometer analysis of the major peaks identified in the phytochemicals of *Solanum lycopersicum* leaf extract (A- N).

Key:
A: Mass spectrum of the peak with RT 3.36 (Bicyclo[3.1.0]hex-3-en-2-one, 4-methyl-1-(1-methylethyl)-)
B: Mass spectrum of the peak with RT 4.25 (γ-Terpinene)
C: Mass spectrum of the peak with RT 5.67 (Terpinen-4-ol)
D: Mass spectrum of the peak with RT 6.71 (Bicyclo[3.1.0]hex-3-en-2-one, 4-methyl-1-(1-methylethyl)-)
E: Mass spectrum of the peak with RT 7.90 (Eugenol)
F: Mass spectrum of the peak with RT 8.90 (Humulene)
G: Mass spectrum of the peak with RT 9.65 (Naphthalene, 1, 2, 3, 4, 4a, 7-hexahydro-1, 6-dimethyl-4-(1-methylethyl)-)
H: Mass spectrum of the peak with RT 10.19 (Caryophyllene oxide)
I: Mass spectrum of the peak with RT 11.43 (1H-3a, 7-Methanoazulene, octahydro-1, 4, 9, 9-tetramethyl-)
J: Mass spectrum of the peak with RT 12.91 (Benzene, 1-(1,1-dimethylethyl)-3-methyl-)
K: Mass spectrum of the peak with RT 13.78 (Thymol)
L: Mass spectrum of the peak with RT 14.09 (Phenol, 2-ethyl-4,5-dimethyl-)
M: Mass spectrum of the peak with RT 15.52 (Dimethyl phthalate)
N: Mass spectrum of the peak with RT 16.16 (Pyrazine, 2, 3-diethyl-5-methyl-)

DISCUSSION

Phytochemicals play a crucial role in the pharmaceutical industry, particularly in the development of therapeutic agents and novel drugs. The discovery of new drugs often begins with the identification of active principles from natural sources. A promising approach to finding therapeutically active compounds involves screening plant extracts from various species. Phytochemicals, such as saponins, alkaloids, flavonoids, terpenoids, and tannins, have been found to exhibit a range of biological properties, including anti-inflammatory, antioxidant, antiulcer, anticancer, and antidiarrheal activities (Ishak *et al.*, 2024).

The present qualitative phytochemical analysis result showed the presence of saponins, phenols, phlobatannins, alkaloids, steroids, and flavonoids while anthraquinones glycosides, cardiac glycosides, tannins, and terpenoids were completely absent. This is in agreement with the work of Mohammed *et al.* (2014) which revealed that flavonoid, saponins and steroids were usually present in healthy *Solanum lycopersicum* leaves.

The presence of alkaloids, flavonoids, phenols, phlobatannins, saponins in the present study is in accordance with previous phytochemicals identified by Ramya *et al.* (2022). The difference observed with the absence of cardiac glycoside could be attributed to some environmental factors where the healthy leaves were collected. Phenols are known for their ability to retard the lipid oxidation in oils, fatty acids and therefore reducing the risk of cardiovascular diseases. Phenols are also noted to interfere in the propagation of cancer cells of various stages, thereby reducing the risk of cancer occurrence in individuals (Benedec *et al.,* 2013). Flavonoids and other phenolic compounds have been widely recognized for their pharmaceutical and medical applications. Studies have linked the antioxidant properties of these compounds to a reduced incidence of various diseases, including cancer. Also, phenolic compounds have been established as effective chemopreventive agents, highlighting their potential in disease prevention and treatment (Tungmunnithum *et al.*, 2018; Karigidi *et al.*, 2020)

The presence of alkaloids and saponins play a role in protecting the plant from various pathogens such as fungi, bacteria and viruses. Alkaloids are considered to be the most efficient therapeutic agent among plants phytochemicals. Purely synthesized alkaloid can be used as medicinal agents because of their analgesic and antibacterial properties (Eleazu *et al.,* 2012). Steroids are also well known for their anti-inflammatory properties (Okey-Nzekwe *et al.*, 2019; Srivastava *et al.*, 2025).

A total of 14 compounds identified by GC-MS analysis in the petroleum ether extract of *Solanum lycopersicum* leaves revealed some antioxidant potentials in tomato. Thymol, Eugenol, y-terpinene, humulene, pyrazine, etc. are few examples of the various types of phytoconstituents (Koch *et al.,* 2012) and a large number of them have given positive results in cancer management, analgesic, antibacterial, antifungal to mention a few.

It has been earlier reported that Solanum plants, which are members of the Solanaceae family, possess anti-cancer properties (Kowalczyk *et al.,* 2022; Manoharan *et al.*, 2024). The current study on *S. lycopersicum* established that the crude leaf extract had abundant phyto-components which possess antioxidant constituents that demonstrate its medicinal potential. Thus, *S. lycopersicum* leaves though not typically consumed by humans, may serve as a potential source for drug formulation, representing a valuable resource beyond mere waste.

Further investigation is recommended to

isolate the effective bioactive components identified and to confirm their biological activities.

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