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PHYTOCHEMICAL PROFILING OF BIOACTIVE METABOLITES IN METHANOLIC EXTRACT OF TWO WILD SOLANUM SPECIES AND EVALUATION OF THEIR ANTIOXIDANT ACTIVITY.

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ABSTRACT

Medicinal plants abound in conventional medicines with antioxidant potentials that may be of value for the treatment of diseases, but with little or no scientific basis for their use. So, the present study aims to conduct a detailed investigation of phytochemicals present in two wild Solanum plants and also their potential use as an effective antioxidant agent. The objective of this present work was to identify the phytochemical compounds by using Gas chromatography–mass spectrometry technique and its biological activity of Solanum plant leaf extracts of methanol solvent systems were predicted using PASS online software. The methanolic leaf extract of *Solanum nigrum* and *Solanum lycopersicum* was tested to determine its chemical composition. The result of the preliminary phytochemical investigation in methanolic extract of Solanum revealed the presence of several bioactive secondary metabolites. In order to better understand the common uses of this plant, the antioxidant activity of the extracts was also measured using three separate assays.: "radical 2,2- diphenyl-1-picrylhydrazyl (DPPH) scavenging activity, Ferric-reducing antioxidant power (FRAP) and 2,2-azino-bis(3-etilbenzotiazolin)-6-sulfonic acid (ABTS) scavenging assay". In different antioxidant assays, the methanolic extract showed different IC50 values slightly higher than the standard to confirm the importance of medicinal plant's bioactive metabolites.

Keywords: GC-MS analysis, PASS, *S. nigrum*, *S. lycopersicum*, phytochemical screening, antioxidant activity

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INTRODUCTION

Almost 80 % of the world's populations use medicinal plants or their metabolites as their primary health care or for treatment Mahomoodally (2013). They already known as a multi-purpose medicinal plant (MMP) and safe for consumption contributes to the availability of a spectrum of medicinal products originating from certain herbs and plants Popoola *et al*. (2020).

Solanum spp has been traditionally used as an emollient, antiseptic, analgesic, antidysentery, tonic, antispasmodic, laxative, antinarcotic, soporific, diuretic, antiulcer, anti-cancer and for disorders of neuro-vegetative system "Saijo *et al*. 1982; Akhtar and Muhammad (1989); Edmonds and Chweya (1997); Manoko *et al*. (2007); El-Shaboury *et al*. (2017)".

Family Solanaceae exhibits A broad variety of secondary metabolites, making them very valuable for agricultural, economic and pharmaceutical purposes "Zadra *et al*. (2012); Mohasana *et al*. (2020)". The per-centages of active constituent in the secondary substances determine the potential of its uses in the preparation of drugs. "Gas Chromatography Mass Spectroscopy (GC-MS)" is useful technique for separation and identification of complicated mixture of metabolites "Witte *et al*. (1987); Hussein *et al*. (2017)." Recently, gas chromatography mass spectroscopy (GC–MS) has been applied to determine the structures of different plant metabolites and other biological samples with great success **"**Anna *et al*. (2017)". In the Egyptian flora, wild medicinal plants are exposed to serious risk due to heavy human impacts such as overgrazing, uncontrolled tourism and unregulated collection, mining and quarrying. Inclusion of wild plant conservation strategies into development plans of the Egyptian economy would minimize human impacts on these plants and ensure their sustainable use in both herbal medicine and pharmaceutical compounds preparations Badr *et al*. (2017). Secondary metabolites with promising biological activity, such as hydroxycinnamic acid amides (HCAAs), polyphenols, and steroid alkaloids, are known to be biosynthesized by Solanaceae, probably to protect themselves from phytopathogenic harm "Ajaiyeoba *et al* (1998); Macoy *et al*. (2015)." The presence of some phytochemical like flavonoids and alkaloids illustrate the medicinal action of the plant encountered in its therapeutic uses "Bamishaiye *et al*. (2011)." Several studies confirmed presence of several amounts of phytochemicals in *Solanum* species such as flavonoids, alkaloids, phenols and tannins "Manish (2012); Akilan *et*

al. (2014); Indra *et al*. (2019), in family Solanaceae Popova *et al*. (2020)."

Antioxidant activity might be resulted from presence of polyphenolic compounds "Upadhyay *et al*. (2015)." The production and releasing of oxygen species (ROS) is a major cause of functional and structural modulation of cell membranes. Free radicals typically attack polyunsaturated fats in mitochondria and cellular membranes begin the uniqe source of ROS when the normal metabolic chain is impeded. In such cases, compound exhibiting antioxidant activity can be valuable in blocking the development of ROS and may also reduce oxidative cell damage, which is completely of interest if this activity is induced by a medicinal food "Lobo *et al* (2010)." ROS are associated in damage to most macromolecules, such as proteins, lipid and DNA, and also cause cell death "Uttara *et al*. (2009)." Plant secondary metabolites can be utilized to regulate the steadiness be-tween free radicals and antioxidants and can be a less dangerous alternative relative to synthetic antioxidants "Godwill (2018) ". Due to several adverse effects produced by synthetic antioxidants, the world now searching for safe therapeutic medicinal plants in the treatment of several diseases related to oxidative stress. As a result, a variety of contemporary studies have concentrated on seeking of promising novel antioxidants, especially flavonoids, and phenolics from natural resources, they have comparable characteristics but is far less poisonous and have clear and significant biological effects.

Plants generally have antioxidant properties, which have a good and various agreement of metabolites, for example, a few classes of flavonoids and non-flavonoids. These metabolites are varied in structure, OH groups, and their position is very essential to deviation in their antioxidant activity and also their biological actions "Wang *et al*. (2018)" Both of Herbs and vegetables especially wild species reach in antioxidant agents and several phytochemicals product is able to postpone oxidative pressure and the other related issues "Forni *et al*. (2019)" Therefore, the present study was designed to detect various phytochemicals present in methanolic extract of *S. nigrum* and *S. lycopersicum* and its use as a strong antioxidant agent.

The present study is an attempt to focus on identification of compounds by using GC-MS technique and biological activities of identified compounds from plant leaf extract to correlate the traditional medicine uses of *Solanum* with composition of extracts obtained from each plant and with their antioxidant activities. Finally, several compounds were isolated from two plant species and their structures were determined and also their antioxidant activities.

MATERIALS AND METHODS

Plant Material collection and Extraction Procedures: The whole wild of *S. nigrum* plants were collected in April 2020 from Fayoum governorate-Egypt, *S. lycopersicum* plant was collected in January 2020 from Ismailia Governorate- Egypt. The collected species washed thoroughly with distilled water. They were blotted with blotting paper, shade dried at room temperature, and ground into a fine powder. 100 gm. of powdered samples were extracted successively with 750 ml. of methanol. Extract was filtered through a 45 µm filter. The resulting solution was concentrated in vacuum to dryness to give methanol extract (5 gm.). The extract was stored at 4°C for use later.

GC-MS investigation: The phytochemical investigation of methanolic extract was performed on a GC-MS equipment "(GC/MS system: shimadza GC/MA-QP5050A, Searched library: Wiley. Software class: 5000. Column DBI : 30m : 0.53 mm ID 1.5 Mm film 9JCW scientific, Ionization model : EL, Carrier gas : helium, Ionization voltage : 70 ev, Temperature program 40 °C (1 min)- 150 °C (1min) at 3.5 °C /min- 250 °C (2min) at 5 °C /min- 270 °C (1min) at 7 °C /min, Injector temperature : 250 °C and Detector temperature : 300 °C."

Identification of compounds was based on the molecular structure, molecular mass.: Mass spectrum data was provided using the database of online Wiley library. Molecular name, size and structure of the compounds of the test material were ascertained by correlating with the library. The biological activities were predicted using PASS online software after obtaining their SMILES by using Open Parser for Systematic IUPAC nomenclature (OPSIN).

Determination of Antioxidant Activity

2,2-diphenyl-1-picryl-hydrazyl-hydrate "DPPH assay": Solutions of the provided samples were prepared in concentrations 1000 and 100µg/mL in methanol in order to identify a range of concentrations within which the inhibitory concentration 50 (IC50) lies. Extracts that surpassed 50% inhibition in in each of the initial screening phase were serially diluted to give 5 concentrations carried out according to the method of Boly *et al*. (2016) . "100µL of freshly DPPH reagent (0.1% in methanol) were added to 100µL of the sample/ compound (dissolved in MeOH) in 96 wells plate (n=3; Values are Mean of 3 replicates), the reaction was incubated at 37 °C. for 30 min in dark". "The resulting decrease in DPPH color intensity was calculated at 540 nm at the end of the incubation period. The data was evaluated using Microsoft Excel® and the value of IC50 was determined."

2,2-Ferric Reducing Antioxidant Power "FRAP assay": Carried out according to the method of "Benzie *et al*. (1996)" but in microplates, briefly; freshly prepared

"TPTZ rea-gent (300 mM Acetate Buffer (PH=3.6), 10 mM TPTZ in 40mM HCl, and 20 mMFeCl3, in a ratio of 10:1:1 v/v/v, respectively)". 190 uL from the freshly prepared TPTZ were mixed with 10 µL of the sample in 96 wells plate (n=3; Values are Mean of 3 replicates), the reaction was incubated at room temp. for 30 min in dark. At the end of incubation time the resulting blue color was estimated at 593 nm at the end of the incubation cycle. Data was evaluated using Microsoft Excel® and ferric sample reduction capability is pre-sent as µM TE/mg.

2,2-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid "ABTS assay": According to Arnao *et al*. (2001) but carried out in microplates; 192 mg of ABTS were dis-solved in distilled water and transferred to 50 mL volumetric flask then the volume was completed with distilled water. 1mL of the previous solution was added to 17 µL of 140 mM potassium persulphate and the mixture was left in the dark for 24 hours. After that, 1mL of the reaction mixture was completed to 50 mL with methanol to obtain the final ABTS dilution used in the assay. 190 µL of the freshly prepared ABTS reagent were mixed with 10 µL of the sample/ compound in 96 wells plate (n=3; Values are Mean of 3 replicates), the reaction was incubated at room temp. for 120 min in dark. The decrease in ABTS color intensity was estimated at 734 nm at the end of the incubation period. The data was evaluated using Microsoft Excel® and the value of IC50 was determined.

RESULTS

Bioactive phytochemicals in the methanolic extract of *S. nigrum* and *S. lycopersicum*: Presence of diverse secondary metabolites has been reported with different structures and types present in *S. nigrum* revealed as different peaks and each one has different relative abundance at different retention times where the highest peak appeared after 18.81 min at figure 1.a, the variety of compound were identified and illustrated in Table 1. In *S. lycopersicum* revealed different peaks and each one has different relative abundance at different times where the highest peak appeared after 18.91 min at figure 1.b, the variety of compound were identified and illustrated in Table 2. The pharmacological activity of compounds varied this showing the importance of plant metabolites.

By GC-MS 27 metabolites identified in *S. nigrum* and 35 in *S. lycopresicum*. Among the 62 studied compounds 15 were found to be the most abundant compounds in the two Solanum species and these compounds were (1) 4-methyl-4-pentyl-3,4 dihydrocoumarin (2) Eupomatidine (3) 5,7-dihydroxy- 4 -pentyl - 3,4 -di hydrocoumarin (4) Isoochiapin B (5) Palmitic acid (6) 3-(5-Bromoindol-3-yl)succinimide (7) 2,4 Dimethyl- 7 -phenyl pyrido[2,3-d]pyrimidin (8) 3- (4 Nitro phenyl quinazolin-4(3H)-one (9) Isoschumanniophytine (10) Isoorientin (11) Stearic acid (12) 10-(M ethoxycarbonyl)-N-acetylcolchinol (13) Noradrenaline tetra TM S (14) Epinephrine- Tetra TM S. and (15) Stearic acid.

Among 27 metabolites identified in *S. nigrum* about 12 metabolites characterize it, they were (1) Octyl phenol isomer (2) Phenol, 4-(1,1,3,3-tetramethylbutyl (3) 2-(Dodecyloxy) ethanol (4) (3,4-Diacetoxybenzylidene)-2-methyloxazol (5) 3-[(1-Hydroxymethyl) propyl] quinazolin (6) Dihydrovallesiachotamine (7) Fluoro metholone (8) phenylylindolizine (9) Indole-3-carboxylic acid, 5-hydroxy-6- 4-methoxyphenyl (10) 9-Octadecenoic acid (11) Dichotine (12) Lucenin 2.

Among 35 metabolites identified in *S. lycopresicum* about 20 metabolites characterize it, they were (1) Cinnamic acid, methyl ester (2) 1-Eicosanol (3) 4-(2-Propen-1-yloxy) benzeneamine (4) 3-cyano-4-methyl-6-ethyl-2(1H)-pyridone (5) Phenol, p- (1,1,3,3-tetramethylbutyl) (6) Ppropiolic acid, 3 - (1-hydroxy-2-isopropyl -5- methyl cyclohexyl) (7) Phenol, p-(1,1,3,3-tetramethylbutyl) (8) Tetracyclo quinone (9) Timolol (10) 2-Propanol, 1-[4-(1,1-dimethylethyl) phenoxy (11) 5,6-Benzoquinoline (12) 9-formylacridine (13) 2-Benzo[f] iso quinoline carbaldehyde (14) Quercetin 7,3,4 trimethoxy (15) 2,4 -Dimethyl -7- phenylpyrido [2,3-d]pyrimidin-5-one (16) D- erythron sphingosine (17) Stearin, 1,3-di (18) 3-(4-Nitrophenyl)quinazolin-4(3H)-one (19) 2,6-Dimethoxy -4- phenyl -7,8- quinolinedione (20) 1,2 Di-o-octa decanoyl ethandiol

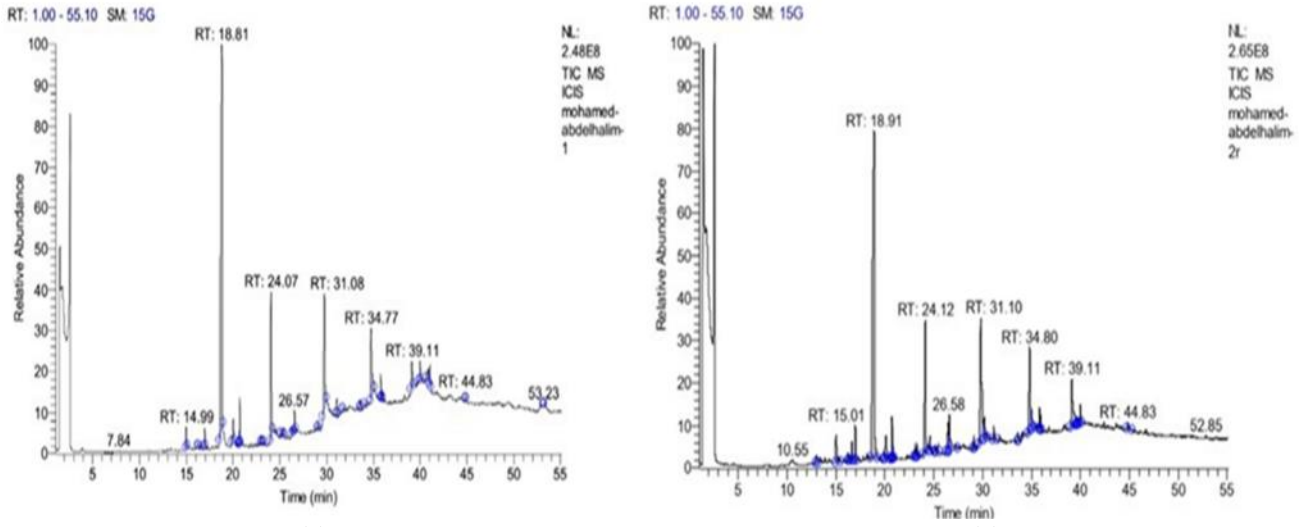
Antioxidant Assay

Free radical scavenging activity in *S. nigrum* and in *S. lycopersicum* by DPPH assay: In *S. nigrum* the DPPH method revealed that the percent of inhibition increase with increasing concentration, where concentration 100 µg/mL yield 44.41± 3.40% inhibition while concentration 800 µg/mL yield 89.32± 1.51%. The relationship between the concentration and percent of inhibition were plotted to determine IC50 (50 % inhibitory concentration) where Ic50 found to be 38.52. (Table 3) Figure 2.a

In *S. lycopersicum* the DPPH method revealed that the percent of inhibition increase with increasing concentration, where concentration 100 µg/mL yield 44.65 ± 2.42 % inhibition while concentration 800 µg/mL yield 88.94 ± 1.51 % (Table 3). The relationship between the concentration and % inhibition was plotted to determine IC50 (50 % inhibitory concentration) where Ic50 found to be 37.29 (Table 3) Figure 2.b.

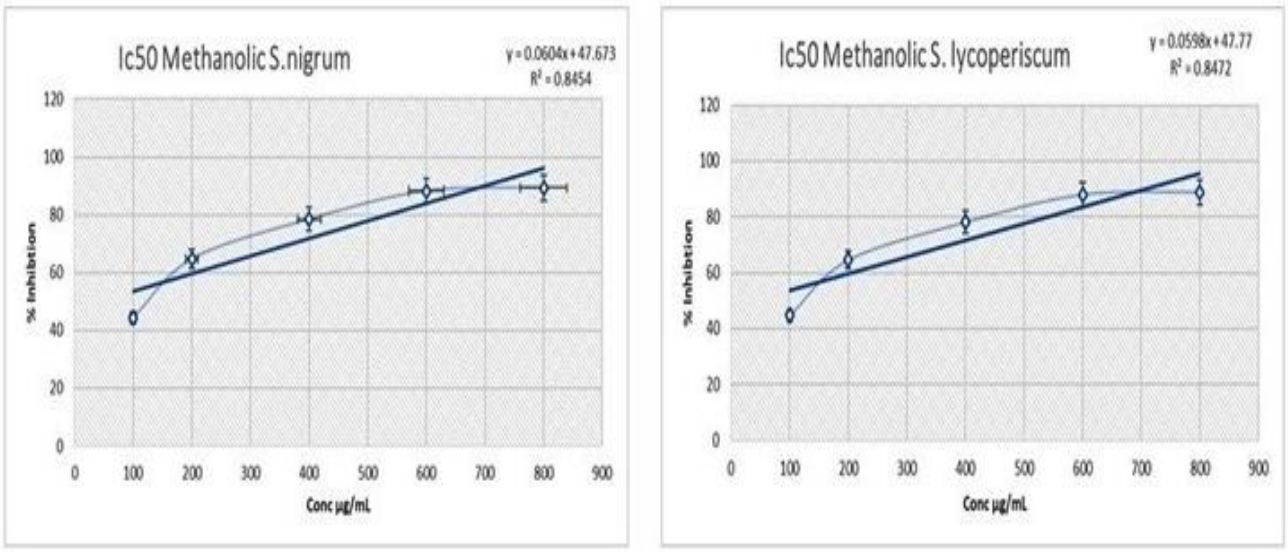
FRAP assay in *S. nigrum* and in *S. lycopersicum*: In *S. nigrum* Ferric reducing ability gave average absorbance at 593 nm = 0.682 ± 0.007), antioxidant activity using FRAP assay is = 132.57 ± 1.41 µMTE / mg, while in *S. lycopersicum* average absorbance at 593 nm = 0.676 ± 0.006), antioxidant activity using FRAP assay is = 131.59 ± 1.21 µMTE / mg. (Table 3).

HRP reducing ability of the *S. nigrum* and in *S. lycopersicum*: In *S. nigrum* average percentage inhibition of ABTS reagent at 734 nm = 65.09 ±0.035%, antioxidant activity using ABTS assay is = 142.63 ± 12.41 µMTE / mg. while in *S. lycopersicum* the average percentage inhibition of ABTS reagent at 734 nm = 0.727±0.035, antioxidant activity using ABTS assay is = 142.50 ± 12.08 µMTE / mg (Table 3).



(a) **(b)**

Figure 1: (a) Relative abundance of methanolic extract revealed by GC-MS analysis of leaves of *S. nigrum*. (b) Relative abundance of methanolic extract revealed by GC-MS analysis of leaves of *S. lycopersicum*.



(a) **(b)**

Figure (2): (a) Free radical scavenging activity at different concentrations of *S. nigrum* methanolic. Values are Mean of 3 replicates. (b) Free radical scavenging activity at different concentrations of *S. lycopresicum* methanolic. Values are Mean of 3 replicates.

Table 1: List of metabolites, their molecular formula, retention time, molecular weight and their biological activities and pharmacological activities identified in *S. nigrum* by GC-MS..

No	Rt	Compound Name	Molecular Formula	Molecular Weight	Biological activities
1	16.94	4-methyl-4-pentyl-3,4dihydrocoumarin	C15H20O2	232	CYP2C12 substrate, Ubiquinol-cytochrome-c reductase inhibitor and Antieczematic
2	18.81	OCTYL PHENOL ISOM ER	C14H22O	206	CYP2C12 substrate,Taurine dehydrogenase inhibitor and Antiseborrheic
3	20.02	"Phenol, 4 - (1,1,3,3-tetramethylbutyl)"	C14H22O	206	Glyceryl-ether monooxygenase inhibitor, Dextranase inhibitor and Phobic disorders treatment
4	20.68	Eupomatidine	C16H10N2O2	262	Antiseborrheic, Antineoplastic (bladder and colon cancer) and Kinase inhibitor
5	20.76	2-(Dodecyloxy)ethanol	C14H30O2	230	Eye irritation inactive, Dextranase inhibitor and Antiseptic
6	23.02	-(3,4-Diacetoxybenzylidene)-2-methyloxazol	C15H13NO6	303	Kinase inhibitor and Aspulvinone dimethylallyltransferase inhibitor.
7	24.07	-5,7dihydroxy-4-pentyl-3,4-dihydrocoumarin	C14H18O4	250	CYP2C12 substrate, Ubiquinol-cytochrome-c reductase inhibitor and Antimutagenic
8	24.07	-3[(1-Hydroxymethyl)propyl]quinazolin	C12H14N2O2S	250	Glycosylphosphatidylinositol phospholipase D inhibitor and Carboxypeptidase Taq inhibitor
9	25.09	ISOCHIAPIN B	C19H22O6	346	Not identified
10	26.32	Palmitic acid	C16H32O2	256	Anesthetic general, Enzymes inhibitor and Antieczematic
11	26.57	Dihydrovallesiachotamine	C21H24N2O3	352	Antileukemic, Antineoplastic and Antiviral (Rhinovirus)
12	29.07	Fluorometholone	C22H29FO4	376	Androgen antagonist, Antiallergic and Antiinflammatory, ophthalmic
13	29.77	3-(5-Bromoindol-3-yl)succinimide	C12H9BrN2O2	292	Anticonvulsant
14	31.08	"2,4 Dimethyl -7- phenylpyrido [2,3-d] pyrimidin"	C15H13N3O	251	Taurine dehydrogenase inhibitor and Glycosylphosphatidylinositol phospholipase D inhibitor
15	34.77	3-(4-Nitrophenyl)quinazolin-4(3H)-one	C14H9N3O3	267	Lysase inhibitor and Glucan endo -1,6- beta-glucosidase inhibitor
16	35.79	phenylylindolizine	C22H17N	295	Glycosylphosphatidylinositol phospholipase D inhibitor and Mucomembranous protector
17	35.79	Isoschumanniophytine	C16H9NO5	295	Anaphylatoxin receptor antagonist, HIF1A expression inhibitor and Antineoplastic
18	35.95	Isoorientin	C27H30O14	578	Cardioprotectant, TP53 expression enhancer and Cytostatic
19	39.11	Stearic acid	C38H74O3	578	Acylcarnitine hydrolase inhibitor and CYP2J substrate
20	39.98	Indole-3-carboxylic acid, 5-hydroxy-6-4-methoxyphenyl	C20H21NO4	339	Antiviral (Influenza) and CYP2H substrate
21	41.07	-9Octadecenoic acid	C38H72O3	576	CYP2J , CYP2J2 substrate and Antieczematic
22	44.83	Noradrenaline tetraTM S	C20H43NO3Si4	457	Antineoplastic
23	44.83	-10(M ethoxycarbonyl)-N-	C22H25NO7	415	CYP2E1 inducer, Leukopoiesis inhibitor and CYP2C9 inducer

		acetylcolchinol			
24	44.83	EPINEPHRINE-TETRATM S	C21H45NO3Si4	471	Cardiovascular analeptic, Respiratory analeptic and Spasmolytic
25	44.83	Dichotine	C25H30N2O9	502	Neurodegenerative diseases treatment, Alzheimer's disease treatment and CYP2H substrate
26	53.15	Lucenin 2	C27H30O16	610	Cardioprotectant, Hepatoprotectant, Antioxidant and Antiviral (Herpes).
27	53.15	Stearic acid	C39H78O3	594	Acylcarnitine hydrolase inhibitor and CYP2J substrate

[1]

Table 2: List of metabolites, their molecular formula, retention time , molecular weight and their biological activities and pharmacological activities identified in *S. lycopresicum* by GC-MS.

No	Rt	Compound Name	Molecular Formula	Molecular Weight	Biological activities
1	12.99	Cinnamic acid, methyl ester	C10H10O2	162	Membrane integrity agonist, Carminative, Insulysin inhibitor and Antihypoxic
2	16.25	-1Eicosanol	C20H42O	298	Sugar-phosphatase inhibitor and Carboxypeptidase Taq inhibitor
3	16.62	-4(2-Propen-1-yloxy)benzeneamine	C9H11NO	149	Respiratory analeptic, Eye and Skin irritation in active
4	16.97	-4M ethyl-4-pentyl-3,4-dihydrocoumarin	C15H20O2	232	CYP2C12, CYP2J substrate and Oxidoreductase inhibitor
5	16.97	-3cyano-4-methyl-6-ethyl-2(1H)-pyridone	C9H10N2O	162	CYP2C12 substrate and Testosterone 17beta-dehydrogenase (NADP+) inhibitor
6	18.91	Phenol, p-(1,1,3,3-tetramethylbutyl)-	C14H22O	206	Glyceryl-ether monooxygenase inhibitor and Nicotinamidase inhibitor
7	19.92	"Ppropiolic acid, 3- (1-hydroxy-2-isopropyl -5-methylcyclohexyl)"	C13H20O3	224	CYP2H substrate, Acylcarnitine hydrolase inhibitor and Carminative
8	20.10	Phenol, p-(1,1,3,3-tetramethylbutyl)-	C14H22O	206	Glyceryl-ether monooxygenase inhibitor and Dextranase inhibitor
9	20.70	Eupomatidine (9-M ethoxynaphtho [2,7]naphthyridin	C16H10N2O2	262	Antiseborrheic and Antineoplastic
10	23.06	Tetracycloquinone	C17H11NO2	261	Complement factor D inhibitor and HIF1A expression inhibitor
11	23.06	Timolol	C13H24N4O3S	316	Adrenaline antagonist, Ophthalmic drug and Cardiotonic
12	23.28	-2Propanol, 1-[4-(1,1-dimethylethyl)phenoxy]	C13H20O2	208	CYP2D2, CYP2C12 Substrates and Anesthetic
13	24.12	-5,7dihydroxy-4-pentyl-3,4-dihydrocoumarin	C14H18O4	250	CYP2C12 substrate and Antimutagenic
14	24.12	-5,6Benzoquinoline	C13H9N	179	Nitrate reductase (cytochrome) inhibitor and NADPH peroxidase inhibitor
15	24.60	-9formylacridine	C14H9NO	207	Aldehyde oxidase inhibitor and Taurine dehydrogenase inhibitor

[1] Rt= Retention time

16	24.60	-2Benzo[f]isoquinolinecarbaldehyde	C14H9NO	207	Antiischemic and Antineoplastic
17	25.11	ISOCHIAPIN B	C19H26O6	350	Not identified
18	26.48	Palmitic acid	C16H32O2	256	Anesthetic general, Enzymes inhibitor and Antieczematic
19	26.56	Quercetin 7,3,4 trimethoxy	C18H16O7	344	Chlordecone reductase inhibitor and Antimutagenic
20	29.81	3 (5-Bromoindol-3-yl) succinimide	C12H9BrN2O2	292	Anticonvulsant
21	30.17	"2,4 Dimethyl-7-phenylpyrido [2,3-d] pyrimidin-5-one"	C15H13N3O	251	Thioredoxin inhibitor
22	31.10	"2,4 Dimethyl-7-phenylpyrido[2,3-d]pyrimidin-5-one"	C15H13N3O	251	Thioredoxin inhibitor
23	31.10	-7Methylthio-2-methyl-5-(4-methoxyphenyl)pyrazolo[1,5-a]pyridine	C16H16N2OS	284	Aspulvinone dimethylallyltransferase inhibitor
24	33.58	Stearin, 1,3-di	C39H76O5	624	All-trans-retinyl-palmitate hydrolase inhibitor and Eye irritation, inactive
25	34.80	-3(4-Nitrophenyl)quinazolin-4(3H)-one	C14H9N3O3	267	Glucan endo-1,6-beta-glucosidase inhibitor
26	34.80	D-erythro-C18-sphingosine	C18H37NO2	299	Sphinganine kinase inhibitor and Angiogenesis stimulant
27	34.98	-2,6Dimethoxy-4-phenyl-7,8-quinolinedione	C17H13NO4	295	Nicotinic alpha4beta4 receptor agonist and JAK2 expression inhibitor
28	35.81	Isoschumanniophytine	C16H9NO5	295	Anaphylatoxin receptor antagonist
29	35.96	Flavone, 5,7-dihydroxy-6c-glucoside	C27H30O14	578	Cardioprotectant, TP53 expression enhancer and Cytostatic
30	39.11	"3,5:6,7-Di-O -cyclopentylidene -D-glycero -D-gulo -heptono-ç-lacton"	C17H24O7	311	L-iduronidase inhibitor and Osmotic diuretic
31	39.65	Stearic acid, 2	C38H74O3	578	All-trans-retinyl-palmitate hydrolase inhibitor and Eye irritation, inactive
32	39.70	1,2 Di-o-octa decanoyl ethandiol	C38H74O4	594	GST A substrate and Saccharopepsin inhibitor
33	44.83	Noradrenaline tetraTM S	C20H43NO3Si4	457	Antineoplastic
34	44.83	-10(M ethoxycarbonyl)-N-acetylcolchinol	C22H25NO7	415	CYP2E1, CYP2C9 inducer and inducer, Leukopoiesis inhibitor
35	44.83	EPINEPHRINE-TETRATM S	C21H45NO3Si4	471	Cardiovascular analeptic, Respiratory analeptic and Spasmolytic

Table (3): Antioxidant activity for *S. nigrum* and *S. lycopersicum* using different antioxidant markers.

Samples	Antioxidant activities		
	DPPH (IC 50)	FRAP (µMTE / mg)	ABTS (µMTE / mg)
S. nigrum	38.52 ±0.25	132.57 ±1.41	142.63 ± 12.41
S. lycopersicum	37.29 ±0.33	131.5 ±1.21	142.50 ±12.08

DISCUSSION

The GC-MS analysis separated all of the metabolites in the examined samples and provided a representative spectral output. Each metabolite ideally produced a specific spectral peak. Retention time also help to discriminate between them. The size of the peaks is equivalent to the quantity of the related compounds in the analyzed specimens. Many medicinal plants have a rich supply of secondary metabolites, such as phenol, alkaloids, flavonoids, glycosides, terpenoids and tannins, determined by GC-MS "Lewis and Ausubel (2006)." There are several and different compounds of alkaloids and phenols identified in *S. nigrum* revealed as different peaks and each one has different relative abundance value at different times where the highest peak appeared after 18.81 min the variety of compound were identified and Relative abundance of methanolic extract revealed by GC-MS analysis of leaves of *S. nigrum* population are almost some of compounds identified have been reported such as phenols and acids in *S. nigrum* "Indra *et al*. (2019)." There are different compounds of alkaloids and phenols present in *S. lycopersicum* revealed as different peaks and each one has different relative abundance at different times where the highest peak appeared after 18.91 min, the variety of compound were identified and illustrated in Table 2, Relative abundance of methanolic extract revealed by GC-MS analysis of leaves of *S. lycoperisicum*.

Similar to our results, "Ammar *et al*. (2017)" have determined the chemical constituents in the bark of *S. verbascifolium* Linn. They have identified a total of 21 phytochemical product in three different extracts from the bark using GC-MS analysis. "El-Shaboury *et al*. (2017)" Identified 116 and 172 compounds extracted at retention time of 5 min, 88 and 138 compounds at retention time of 10 min,186 and 196 compounds extracted at retention time of 25 min and two compounds extracted at the retention time of 30 min. "Indra *et al*. (2019)" determined the essential chemical constituents in the bark of *S. nigrum*. They have identified a total of 43 phytocompounds using GC-MS analysis. In present study they were 27 and 35 compounds were identified for *S. nigrum* and *S. lycopersicum* respectively. The ratio of active constituent determines the potential of its uses for drug preparation and this is elucidated in all extracted metabolites and this is great evidence that medicinal plant metabolites are very excellent and potential source for drug preparation and treatment of diseases "Deepak and Gopal (2014)."

Oxidative stress is the main reason in a many of human disorders, including inflammation, carcinogenesis, neurodegenerative diseases, and aging. Although there are many determinants contribute to the emergence of these diseases, presumed experimental evidence links the production of ROS to biological damage, which can eventually provide a mechanistic foundation for treatment "Nistico *et al*. (2013); Schieber and Chandel (2014); Del Río (2015); Moloney *et al*. (2018); Campisi *et al*. (2019)"

Recently, there seems to be a substantial influence in the health impacts of several natural products, as well as in the in vivo protective function of natural antioxidants derived from medicinal plants or included in dietary food against oxidative damage caused by ROS "Muscoli *et al*. (2014); Loffredo *et al*. (2017); Rizzo *et al*. (2017); Carresi *et al*. (2018); Sharma *et al*. (2018)." In the present study, the antioxidant activity of extracts of plants were assessed using three different methods "(DPPH scavenging activity, FRAP assay and ABTS)" of methanolic extracts at different concentrations (200-1000 µg/mL). Trolox reagent was used as a standard and its activity was evaluated under the same conditions as the extracts revealed that the IC50 of standard was slightly lower than the sample indicate its efficacy in scavenging free radicals, have Powerful antioxidant activity, With fascinating insights for their future valorization as pharmaceuticals. The DPPH technique is based on the measurement of antioxidant scavenging potential towards the stable DPPH radical. This method offers a quick and easy to evaluate the antiradical activities of antioxidants, as the radical compound is stable and would not need to be generated as in other radical scavenging techniques "Zhang *et al*. (2010)". The FRAP method is based on the ability of an antioxidant to transfer an electron for reduction to any compound, such as carbonyl groups, metals and radicals "Brand *et al*. (1995)". ABTS was generated in situ in the presence of antioxidants in the lag-time assay "Benzie *et al*. (1999)".

The scavenging activity of free radical is measured by the ability to quench the stable DPPH radical. The assay gave data on the reactivity of examined compounds with a stable free radical. *S. nigrum* and *S. lycopersicum* extracts were able to quench the DPPH-radical and had nearly equivalent ability. The two extracts content have different level of phenolic and alkaloids compounds, but phenolic and flavones are not significantly different. Only Timolol and Quercetin are

more abundant in *S. lycopersicum*. Similar results obtained by "Campisi *et al*. (2019) " which assess the antioxidant effect of two leave extracts of *S. nigrum* L. and the results showed that the extracts were able to quench the radical and restore the oxidative status in in vitro.

It can be inferred that the therapeutic effects of medicinal plants are usually attributed to their phytochemicals especially phenols and flavonoid; in our extract both of phenol and flavonoid are abundant and are the major cause for antioxidant activity. Phenols play important roles in plants, such as protection against insect pathogens and herbivores. Both of phenols and flavonoids are involved in cementing the material linking phenolic polymers to cell wall polysaccharides "Graham *et al*. (1994); Ilyasov *et al*. (2018)." Flavonoids are the most com-mon and important group of naturally occurring phenolic, because of their wide range of functions. Flavonoids generally act through a chelation or scanning. Flavonoids act as antioxidants by splitting radical chains in more stable products in the membranes of liver microsomes. They also play an significant role in protection against oxidative stress "Oriakhi *et al*. (2014); Martial-didier *et al*. (2017); Nea *et al*. (2021)."

Generally, antioxidants (vitamins C-, E, carotenoids, and polyphenols) are important for good bone health. They neutralize reactive particles called free radicals that are associated with all inflammatory and painful phenomena "Arulselvan *et al*. (2016); Nea *et al*. (2021)."

Recommendation: The therapeutic properties of medicinal plants can be attributed to their phytochemicals, which are the primary cause of both antioxidant activities through quenching different radicals such as DPPH, FRAP and ABTS.

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