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Unveiling the antioxidant and antibacterial potential of aqueous extract of *Solanum indicum* parts (leaves, root and stem) – An *in vitro* study

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ABSTRACT
Solanum indicum (Solanaceae) is widely used in folk and traditional system of medicine to treat various diseases. The present study aims to evaluate the antioxidant, free radical (DPPH) scavenging, in vitro anti-inflammatory and antimicrobial potential of aqueous extract of various parts (leaves, root and stem) of Solanum indicum. The possible bioactive components in the aqueous extract of various parts of Solanum indicum was also analysed by GC-MS. The aqueous extract of Solanum indicum root shows the highest content of phenols, tannins, flavonoids and vitamin C when compared with leaf and stem extracts. The high antioxidant status of the root extract was reflected in the DPPH scavenging potential and HRBC membrane stabilizing assay. Totally 11, 10 and 9 compounds were identified by GC-MS in the aqueous extract of leaf, root and stem respectively in Solanum indicum. The results of antibacterial activity against Escherichia coli and Klebsiella pnuemoniae show that the aqueous extract of Solanum indicum stem was very effective. Though the root part of Solanum indicum is widely used, the results of the present study show that leaves and stem also have antioxidant, anti-inflammatory and antimicrobial activities. Especially the stem part of Solanum indicum shows the presence of compounds with potent antimicrobial property suggesting a possible application in pharmacognosy.

Keywords: *Solanum indicum*, Phytochemicals, Antioxidants, Free radical scavenging potential, *In vitro* anti-inflammatory, Anti-bacterial activity, GC-MS analysis

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INTRODUCTION

Excess of reactive oxygen/nitrogen species (superoxide anion radical, nitric oxide, hydroxyl radicals, hydrogen peroxide etc.,) disturbs cellular homeostasis. These radicals induce oxidative stress by being responsible for protein denaturation, DNA damage, mutagenesis, lipid peroxidation in the cells. Free radical-induced stress is the prime factor for early aging and the pathogenesis of cardiovascular diseases, cancer, and diabetes [1], and inflammatory diseases like rheumatoid arthritis. The oxidative damage caused by these free radicals can be alleviated by antioxidants. Generally, antioxidants function directly by scavenging the free radicals, quenching the singlet-oxygen formation and forming complexes of pro-oxidant metal ions [2] and could prevent oxidative damages. Butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tert-butylhydroquinone and propyl gallate are some of the synthetic antioxidants used to fight against oxidative stress [3].

Artificial antioxidants cause adverse toxic and side effects leading to heart, liver, kidney, and gastrointestinal problems [4]. So search for plant-based antioxidants is always a concern. Throughout the history of human civilization, medicinal plants always played a significant role in human life. India has more than 18,000 higher plant species of which one-third are medicinally important [5]. 11% of the drugs listed by World Health Organisation (WHO) as essential were derived from plant sources and even the precursors for synthetic drugs are plant-derived molecules [6]. Fruits, vegetables, legumes, and whole - grains offer health - promoting benefits due to the synergistic effect of their complex array of phytoconstituents, vitamins, and minerals. These phytochemicals act as antioxidants, antimicrobials, and anti-inflammatory agents. Flavonoids, phenols, and alkaloids are some promising plant-derived potential natural agents to combat diseases and maintain health.

Indigenous systems of medicine always focused on the management of diseases by plant-derived products. The *Solanaceae* family is one of the largest in Angiosperms and comprises more than 1500 species [7]. Genus Solanum has gained more attention due to its notable medicinal importance in the

traditional system of medicine. *Solanum indicum* (Family: *Solanaceae;* in Tamil - Puttiriccuntai, Papparamulli; Mullamkatti) also known as "Brihati / Indian nightshade" is a pricky busy herb distributed throughout tropical and subtropical countries [8, 9]. *Solanum indicum* is widely used in folk and traditional system of medicine to treat various diseases like asthma, painful bronchitis, cardiac troubles, leucoderma, fever, pruritus, and vomiting [9].

Many tribal communities still rely on plants for their primary health care system. Irulas in Hasanur Hills (Southern Western Ghats) consume cooked unripe fruits of *Solanum indicum* to expel tapeworms [10], Oraon communities in Latehar district (Jharkhand) consume the fruits as vegetables [11] and in Kutch District, Gujarat they use *Solanum indicum* to treat gastrointestinal disorders [12]. In Chinese folk medicine, *Solanum indicum* was used to treat breast cancer, rhinitis, and cough [13]. *Solanum indicum* has been used as folk medicine in Taiwan against inflammation, wound infection, ascites, edema, and toothache [14].

Many traditional practitioners use the fruits, leaves, and root of *Solanum indicum* to treat various ailments like rhinitis, cough, sore throat and hiccup, sexual disorders, abdominal pain, and worm infestation, pain and fever, inflammation, insomnia, urinary complications, loss of appetite and anorexia, blood disorders [15]. Roots of *Solanum indicum* form an important ingredient of the Laghupanchamoola category in Dasamoola [16]. An infusion known as Dasamulakvatha possesses medicinal properties like anti-inflammatory, hypoglycemic, antihypertensive, vasodilator, analgesic, anti-cholesterolemia, antirheumatic, antioxidant, and anti-fever medicinal properties bestowed by roots of the ten plants in Dasamoola [17].

Phytoconstituents like polyphenols, flavonoids, alkaloids, saponins, fatty acids, steroidal glycosides, and steroidal glycoalkaloids are reported in *Solanum indicum* [18, 19]. Leaves of *Solanum indicum* have shown 80% mortality against plant-parasitic nematode [20]. Fruit extracts of *Solanum indicum* were evaluated for hepatoprotective [21], antimicrobial [22], antioxidant and depressant activities [23]. *Solanum indicum* leaves were found to possess antibacterial activity [24, 25]. Even though all the parts of *Solanum indicum* like the root bark, stem, and leaves are used by traditional healers, no proper scientific reports are available to validate its medicinal uses. To bridge this lacuna, the present study examined the phytochemical, free radical scavenging, and antibacterial potential of leaf, stem, and root (aqueous extract) parts of *Solanum indicum*.

MATERIALS AND METHODS

Chemicals

Gallic acid, Quercitin, 1,1 diphenyl-2-picryl-hydrazyl (DPPH), ascorbic acid, and dinitro phenyl hydrazine (DNPH) were obtained from Sigma-Aldrich (St. Louis, M.O., USA); Muller Hinton agar from Himedia. All the chemicals and solvents used were of analytical grade.

Collection of plant material, Processing, and extraction

The plant material *Solanum indicum*, used in this investigation was collected from Gummidipondi, Thiruvallur, Chennai, Tamil Nadu. *Solanum indicum* was taxonomically authenticated by Prof. P. Jayaraman (Director, Institute of Herbal Botany, Plant Anatomy Research Centre, Tambaram) Chennai, India. A voucher specimen of *Solanum indicum* was preserved in the Department for future references (PARC/2017/3543). The entire plant was washed in running water to remove the dust and soil particles. The leaves, roots, and stem portions of *Solanum indicum* were separated and dried thoroughly in shade. The dried samples were coarsely ground using a blender and extracted with distilled water using soxhlet extractor. The obtained aqueous extract of leaf, root, and stem parts of *Solanum indicum* was evaporated to dryness and was used for further analysis.

Qualitative Phytochemical analysis

Aqueous extract of leaf, root, and stem parts of *Solanum indicum* were subjected to preliminary phytochemical screening for the identification of its active constituents [26, 27].

Determination of in vitro antioxidants

The aqueous extract of leaf, root, and stem parts of *Solanum indicum* was dissolved in suitable solvents and used for phenol, tannin, flavonoid, and Vitamin C determination.

Total phenol content

The total phenolic content in the extracts was determined using Folin-ciocalteau reagent with gallic acid as the standard by following the method of McDonald et al. [28]. The colour developed was measured spectrophotometrically at 765nm.

Total tannin content

The amount of tannins in the aqueous extract of leaf, root, and stem parts of *Solanum indicum* was determined using Folin-ciocalteau reagent as per the procedure described by Schanderl et al. [29] and the colour developed was measured at 640nm.

Total flavonoid content

The total flavonoid content in the aqueous extract of leaf, root and stem parts was determined using Quercetin as standard [30]. The colour developed was measured at 415nm.

Vitamin C content

The amount of ascorbic acid presents in the aqueous extract of leaf, root and stem parts of *Solanum indicum* was estimated using DNPH reagent [31]. The yellow colour developed was read at 540nm.

Determination of antioxidant activity - DPPH radical scavenging assay

The antioxidant activity of the aqueous extract of leaf, root, and stem parts of *Solanum indicum* was determined using 1,1 diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging assay described by Shimada et al. [32]. The absorbance was measured at 517nm with ascorbic acid as positive control and the concentration of the extract required to scavenge 50% of the radical was calculated (IC_{50}).

Determination of in vitro anti-inflammatory activity - Assay of membrane stabilization (HRBC)

The Human Red Blood Corpuscles (HRBC) assay was performed using Alseiver's solution [33, 34]. Diclofenac sodium served as reference drug. The absorbance was measured at 560nm. The inhibition of hemolysis by the extracts were expressed in %.

Gas chromatography – Mass Spectrometry analysis (GC-MS)

The compounds in the aqueous extract of leaf, root, and stem parts of *Solanum indicum* were analyzed by using GC-MS (Agilent: GC: (G3440A) 7890A. MS: 7000 Triple Quad GCMS) fitted with DB5 MS (30mm × 0.25mm × 0.25 µm) column. Helium (99.99%) was used as carrier gas at a flow rate of 1ml/min. The oven temperature was programmed from 50°C to 170°C at an increase of 40°C/min and then at 10°C/min to 310°C (isothermal for 10min). The injector temperature was 280 °C and 100 µl was the injection volume of the sample. The ion-source temperature was 280 °C and the mass spectra were taken at 70eV. The mass spectrum obtained was interpreted using the National Institute of Standard and Technology (NIST) library.

***In vitro* antimicrobial activity**

The antibacterial activities of the extracts were tested against *Escherichia coli* (MTCC 443) and *Klebsiella pneumonia* (MTCC 109) by well diffusion method [35]. Antibiotic Azithromycin (30µg/ml) served as positive control and DMSO alone served as a negative control. The Muller Hilton agar plates were incubated at 37°C for 24 hours and the antibacterial activity of the extract was determined by measuring the diameter of the zone of inhibition around the well using an antibiotic zone scale.

Statistical analysis

All the tests were performed in triplicates and results were expressed as Mean ± Standard error mean (SEM). The Statistical analysis was performed using SPSS software (Version 12.0).

RESULTS

Percentage yield and qualitative analysis of *Solanum indicum* extracts

The aqueous extraction yield of leaves, root, and stem parts of *Solanum indicum* is presented in Table 1. Maximum yield (25.35%) was obtained with leaves then followed by root (9.51%) and stem (8.78%) aqueous extracts of *Solanum indicum*. The preliminary phytochemical analysis of the aqueous extracts of various parts (leaves, root, and stem) of *Solanum indicum* confirms the presence of phenols, reducing sugars, flavones, glycosides, saponins, alkaloids, proteins, tannins, anthraquinones, quinines, and terpenes in variable quantities (Table 2).

Quantitative estimation of in vitro phytoconstituents

The concentration of the phenolic compounds were found in the following order, root (126.15 ± 5.9 mg / g extract) > leaves (63.12 ± 0.78 mg / g extract) > stem (54.06 ± 0.94 mg / g extract) aqueous extract of *Solanum indicum* (Table 3). The highest amount of tannins was obtained in aqueous root extract of *Solanum indicum* (34.04 ± 1.93 mg/g extract) when compared with stem and leaf extracts (Table 3). The aqueous stem extract of *Solanum indicum* reported lowest amount of flavonoid (4.23 ± 0.12 mg/ g extract) followed by leaf extract (7.87 ± 0.35 mg/ g extract) and root extract (9.07 ± 0.29 mg/ g extract). The root extract of *Solanum indicum* was rich in Vitamin C content (21.27 ± 1.51mg/g extract) followed by leaf extract (9.66 ± 0.42 mg/g extract), and stem extract (5.10 ± 0.56 mg/g extract) (Table 3).

***In vitro* DPPH free radical scavenging and anti-inflammatory capacity**

The free radical, DPPH scavenging ability/antioxidant capacity of the three aqueous extracts (leaf, root and stem) of *Solanum indicum*, and the positive control ascorbic acid is depicted in Table 4. The radical scavenging ability of ascorbic acid is IC_{50} 71 ± 3.86 µg/10µl. Among the three extracts, the root extract of

Solanum indicum scavenged better with a low IC_{50} (135.46 ± 1.88 µg/10µl) value when compared with leaf extract ((IC_{50} 342.22 ± 4.97 µg/10µl) and stem extract (IC_{50} 361.31 ± 3.86 µg/10µl) of *Solanum indicum*.

The ability of the three aqueous extracts (leaf, root, and stem) of *Solanum indicum* to protect the RBC membrane was determined by membrane stabilization assay. Diclofenac sodium, the positive control offered 89.2 ± 1.50 % protection against hemolysis (Table 4). The order of the inhibition of hemolysis was found to be root extract (44.56 ± 1.40 %) > leaf extract (37 ± 1.62 %) > stem extract (30.76 ± 2.6 %) of *Solanum indicum*.

GC- MS analysis of *Solanum indicum* extracts

The full scan gas chromatogram of active molecules present in the aqueous extract of *Solanum indicum* leaves is given in Figure (1). Eleven active compounds were detected in the aqueous leaf extract of *Solanum indicum* (Table 5). Based on the peak area of individual active compounds, the predominant compounds were catechol (25.02%), tetraacetyl-d-xylonic nitrile (17.78%), 2-pyrrolidin-1-yl-bicyclo[3.3.1]nonan-9-ol (16.03%), paromomycin (12.05%), phenol, 3-methyl-5-(1-methylethyl)-methylcarbamate (8.98%), acetamide, n-methyl-n-[4-(3-hydroxypyrrolidinyl)-2-butynyl]-(6.64), folic acid (3.9%), 2-myristynoyl pantetheine (2.77%), 5,8,11,14-eicosatetraenoic acid, methylester,(all-z)-(2.82%), adenosine-3-phosphoric acid (1.93%) and pyrrolizin-1,7-dione-6-carboxylic acid, methyl (ester) (1.84%).

The components present in the aqueous root extract of *Solanum indicum* analysed by GC – MS analysis are shown in Figure 2 and Table 6. In the root extract, the compound α-D-Glucopyranoside, O- α-D-glucopyranosyl-(1.fwdarw)-β-D-fructofuranosyl covered the maximum peak area 23.89% followed by Catechol (21.62%), 4H-pyran-4-one, 2,3, dihydro-3,5-dihydroxy-6-methyl- (18.54%), Tetraacetyl-d-xylonic nitrile (17.26%), Paromomycin (4.91%), Bicylo(3.3.1) nonan-9-one, 2-(1-pyrrolidinyl)- (4.86%), Ethanone, 1-(2-hydroxy-5-methylphenyl)- (4.68%), Acetamide, N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl]- (1.68%), - 1H-6-purinone,6,7-dihydro-2-amino-7[3,5,-dihydroxy-6-(hydroxymethyl) tetrahydro-2H-2-pyranyl (1.45%) and Columbin (1.1%).

GC – MS analysis of aqueous stem extract of *Solanum indicum* exhibits peaks for 9 compounds (Figure 3 and Table 7). These compounds were as follows with their area (%), paromomycin (30.35%), tetraacetyl-d-xylonic nitrile (20.19%), bicylo(3.3.1) nonan-9-one, 2-(1-pyrrolidinyl)- (15.48%), cyclohexanone (8.58%), acetamide, N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl]- (7.36%), 1- heptatriacotanol (5.47%), olecic acid (5.07%), 5,8,11,14-eicosatetraenoic acid, methylester,(all-z)-(4.59%) and cyclopropanebutanoic acid- 2[[2-[[2-[(2-pentylcyclopropyl)methyl] cyclopropyl]methyl] cyclopropyl] methyl] -,methyl ester (2.91%).

***In vitro* antibacterial activity**

The antibacterial activity of the aqueous extracts of leaves, root, and stem parts of *Solanum indicum* against *Escherichia coli* and *Klebsiella pneumonia* is mentioned in Table 8 and Figure 4. Root and stem aqueous extracts of *Solanum indicum* have shown better activity against *Escherichia coli* when compared with aqueous leaf extract of *Solanum indicum*. Both root (Zone of Inhibition (ZOI) = 13.06 ± 0.23 mm at 100µg/well) and stem extract (ZOI = 14.13 ±0.18 mm at 100µg/well) have shown a dose-dependent increase in the activity against *Escherichia coli*. The activity of aqueous leaf extract against *Klebsiella pneumoniae* was less (ZOI = 4± 0.1 mm at 100µg/well) when compared with root (ZOI = 8 ± 0.05 mm at 100µg/well) and stem (ZOI = 10.1 ± 0.05 mm at 100µg/well) extracts of *Solanum indicum*. The positive control Azithromycin (30µg/well) effectively inhibits *Escherichia coli* ((ZOI = 17.2 ± 0.02 mm) and *Klebsiella pneumonia* (ZOI = 13.2 ± 0.05 mm).

Table 1: Total yield percentage of leaf, root and stem of *Solanum indicum*

Extract Part	Yield (% w/w)
Leaf	25.355
Root	9.517
Stem	8.789

Table 2: Phytochemical analysis of aqueous extracts of Leaf, root and stem *of Solanum indicum*

S. No	Phytochemical / Plant Part	Leaf	Root	Stem
1	Phenols	+++	+++	+++
2	Reducing sugars	+	+++	++
3	Flavones	++	++	+
4	Glycosides	+	+	+
5	Saponins	+++	+++	+++
6	Alkaloids	+++	+++	+++
7	Anthraquinones	++	+++	+++
8	Quinones	+	+++	++
9	Proteins	+	+	+
10	Tannins	++	+++	++
11	Steroids	+	+	+
12	Terpenes	+++	++	+

(+) mild; (++) moderate; (+++) high; (–) not present

Table 3: Amount of phenols, tannins, flavonoids and Vitamin C in aqueous extracts of leaf, root and stem of *Solanum indicum*

S.No	Extract part	Phenols (mg / gm extract)	Tannins (mg / gm extract)	Flavonoids (mg / gm extract)	Vitamin C (mg / gm extract)
1	Leaf	63.12 ± 0.78	22.92 ± 0.95	7.87 ± 0.35	9.66 ± 0.42
2	Root	126.15 ± 5.9	34.04 ± 1.93	9.07 ± 0.29	21.27 ± 1.51
3	Stem	54.06 ± 0.94	25.14 ± 0.43	4.23 ± 0.12	5.10 ± 0.56

Values are expressed as Mean ± SEM (n=3)

Table 4: DPPH scavenging and invitro anti-inflammatory potential of aqueous extracts of leaf, root and stem of *Solanum indicum*

S.No	Extract part / Standard	DPPH (IC_{50}=µg/10µl)	Prevention of hemolysis (%)
1	Leaf	342.22 ± 4.97	37.00 ± 1.62
2	Root	135.46 ± 1.88	44.56 ± 1.4
3	Stem	361.31 ± 2.66	30.76 ± 2.6
4	Standard Ascorbic acid Diclofenac Sodium	55.71±3.86 ---------	----- 89.02 ± 1.50

Values are expressed as Mean ± SEM (n=3)

Table 5: Compounds identified in the aqueous leaf extract of *Solanum indicum*

S.no:	Retention time (RT)	Name of the compound	Percentage of peak area
1	3.83	Paromomycin	2.77
2	3.95	2-myristynoyl pantetheine	3.01
3	4.01	Catechol	25.02
4	4.08	Folic acid	3.9
5	4.16	Paromomycin	3.86
6	4.34	2-pyrrolidin-1-yl-bicyclo[3.3.1]nonan-9-ol	16.03
7	4.49	Phenol, 3-methyl-5-(1-methylethyl)-, methylcarbamate	8.98
8	4.63	Paromomycin	2.43
9	5.79	Pyrrolizin-1,7-dione-6-carboxylic acid, methyl (ester)	1.84
10	6.02	Tetraacetyl-d-xylonic nitrile	17.78
11	6.23	5,8,11,14-eicosatetraenoic acid, methylester,(all-z)-	2.82
12	6.56	Paromomycin	2.99
13	6.77	Acetamide, n-methyl-n-[4-(3-hydroxypyrrolidinyl)-2-butynyl]-	2.47
14	7.04	Adenosine-3-phosphoric acid	1.93
15	7.14	Acetamide, N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl]-	4.17

Table 6: Compounds identified in the aqueous root extract of *Solanum indicum*

S.no:	Retention time (RT)	Name of the compound	Percentage of peak area
1	3.75	Tetraacetyl-d-xylonic nitrile	1.84
2	3.82	4H-pyran-4-one, 2,3, dihydro-3,5-dihydroxy-6-methyl-	18.54
3	4.00	Catechol	21.62
4	4.19	Tetraacetyl-d-xylonic nitrile	2.87
5	4.25	Paromomycin	1.04
6	4.29	Bicylo(3.3.1)nonan-9-one, 2-(1-pyrrolidinyl)-	2.72
7	4.36	Paromomycin	2.94
8	4.49	Ethanone, 1-(2-hydroxy-5-methylphenyl)-	4.68
9	4.63	Paromomycin	0.93
10	4.81	Acetamide, N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl]-	0.52
11	4.86	1H-6-purinone,6,7-dihydro-2-amino-7[3,5,-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-2-pyranyl	1.45
12	5.12	α-D-Glucopyranoside, O- α-D-glucopyranosyl-(1.fwdarw)-β-D-fructofuranosyl	23.89
13	6.02	Tetraacetyl-d-xylonic nitrile	11.32
14	6.32	Tetraacetyl-d-xylonic nitrile	1.23
15	6.77	Acetamide, N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl]-	1.16
16	6.86	Bicylo(3.3.1)nonan-9-one, 2-(1-pyrrolidinyl)-	2.14
17	8.37	Columbin	1.1

Table 7: Compounds identified in the aqueous stem extract of *Solanum indicum*

S.no:	Retention time (RT)	Name of the compound	Percentage of peak area
1	3.82	Paromomycin	3.14
2	4.03	Bicyclo(3.3.1)nonan-9-one,2-(1-pyrrolidinyl)-	15.48
3	4.34	Paromomycin	11.17
4	4.49	Cyclohexanone	8.58
5	4.63	Paromomycin	3.39
6	5.09	Paromomycin	8.64
7	6.07	Tetraacetyl-d-xylonic nitrile	17.22
8	6.23	Tetraacety-d-xylonic nitrile	2.97
9	6.77	Acetamide, N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl]-	3.86
10	6.85	Acetamide, N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl]-	2.29
11	7.06	Paromomycin	1.17
12	7.45	1-Heptatriacotanol	1.58
13	7.78	5,8,11,14-Eicosatetraenoic acid, methylester,(all-z)-	1.48
14	8.15	Cyclopropanebutanoic acid, 2[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]-,methyl ester	2.91
15	8.37	Acetamide, N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl]-	1.21
16	8.85	Paromomycin	2.84
17	9.07	5,8,11,14-Eicosatetraenoic acid, methylester,(all-z)-	3.11
18	9.34	1-Heptatriacotanol	3.89
19	9.58	Oleic acid	5.07

Table 8: Activity of Phytoconstituents identified in all (leaf, root and stem) extracts of *Solanum indicum*

S.no:	Name of the compound	Bioactivity
1	Paromomycin	Antimicrobial activity [49]
2	2-myristynoyl pantetheine	-----
3	Catechol	Antioxidant, pharmaceuticals and cosmetics [53,54] As estrogen backbone, stimulant [51]
4	Folic acid	Synthesis of Hemoglobin and maintenance of mental and emotional health [57]
5	2-pyrrolidin-1-yl-bicyclo[3.3.1]nonan-9-ol	-----
6	Phenol, 3-methyl-5-(1-methylethyl)-, methylcarbamate	Oligosaccharide provider, Catechol O methyl Transferase Inhibitor, methyl donor, methyl guanidine inhibitor [51]
7	Pyrrolizin-1,7-dione-6-carboxylic acid, methyl (ester)	Anti-viral and anti-Tumor activity [50]
8	Tetraacetyl-d-xylonic nitrile	Anti-oxidative activities and anti-viral effects [50] 17 beta hydroxysteroid dehydrogenase inhibitor, alcohol dehydrogenase inhibitor, anticancer, antidote, CNS depressant, coronary dialator, and decongestant [51]

9	5,8,11,14-eicosatetraenoic acid, methylester,(all-z)-	Antifungal, antibacterial, antitumor cytotoxic effects [55]
10	Acetamide, n-methyl-n-[4-(3-hydroxypyrrolidinyl)-2-butynyl]-	Anti-inflammatory activities [52]
11	Adenosine-3-phosphoric acid	-----
12	4H-pyran-4-one, 2,3, dihydro-3,5-dihydroxy-6-methyl-	Insecticidal property [58] Antimicrobial, Anti inflammatory [59]
13	Bicylo(3.3.1)nonan-9-one, 2-(1-pyrrolidinyl)-	Aids in treatment of stomach pain [56]
14	Ethanone, 1-(2-hydroxy-5-methylphenyl)-	-----
15	1H-6-purinone,6,7-dihydro-2-amino-7[3,5,-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-2-pyranyl	-----
16	α-D-Glucopyranoside, O- α-D-glucopyranosyl-(1.fwdarw)-β-D-fructofuranosyl	Anti-diabetic activity [50]
17	Columbin	
18	Cyclohexanone	Antibacterial activity [60]
19	1-Heptatriacotanol	Anti-microbial [61] Enzyme inhibitor and anti-hypercholesterolemic effects [62]
20	Cyclopropanebutanoic acid, 2[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]- ,methyl ester	-----
21	Oleic acid	Acidifier, Arachidonic acid inhibitor, inhibit production of uric acid, increase aromatic amino acid decarboxylase activity [51]

Table 9: Antibacterial activity of aqueous extracts of leaf (L), root (R) and stem (S) of *Solanum indicum* against *Escherichia coli* and *Klebsiella pneumoniae* (Zone of inhibition (ZOI) in mm) and Azithromycin as standard.

Extract Parts / Standard drug	Concentration (µg/well)	Zone of inhibition (mm)	
		Escherichia coli	*Klebsiella pneumoniae*
Leaf	20	0	0
	50	5.43 ± 0.28	0
	75	6.9 ± 0.12	3.13 ± 0.08
	100	8 ± 0.17	4.1 ± 0.1
Root	20	8.93 ± 0.06	3.2 ± 0.11
	50	10.9 ± 0.1	5.03 ± 0.03
	75	12.03 ± 0.20	7.13 ± 0.05
	100	13.06 ± 0.23	8 ± 0.05
Stem	20	11.1 ± 0.15	7 ± 0
	50	12.1 ± 0.1	8.13 ± 0.05
	75	13 ± 0	9 ± 0.05
	100	14.13 ± 0.18	10.1 ± 0.05
Azithromycin	30	17.2 ± 0.02	13.2 ± 0.15

Values are expressed as Mean ± SEM (n=3)

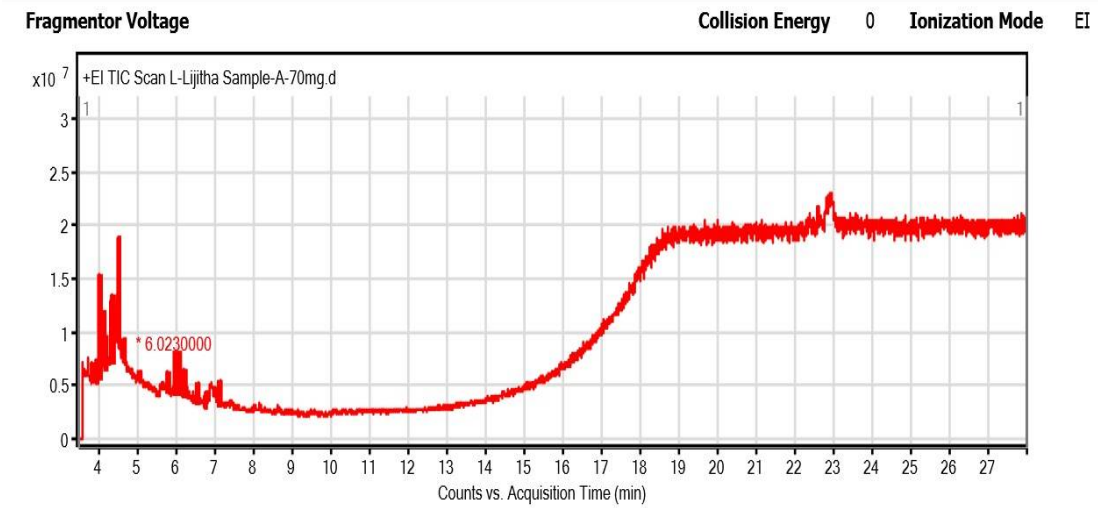


Figure 1: Chromatogram of the aqueous leaf extract of *Solanum indicum*

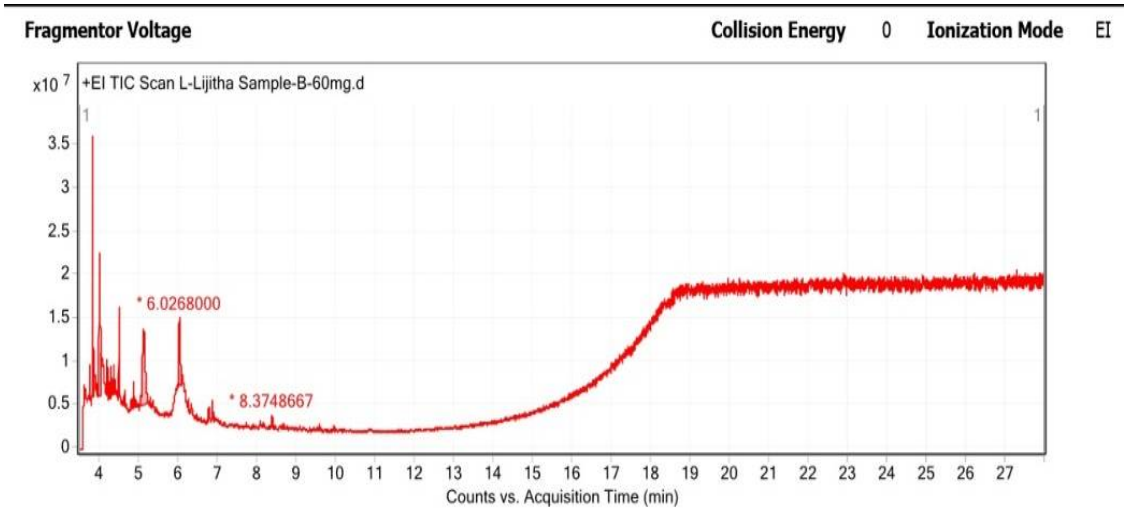


Figure 2: Chromatogram of the aqueous root extract of *Solanum indicum*

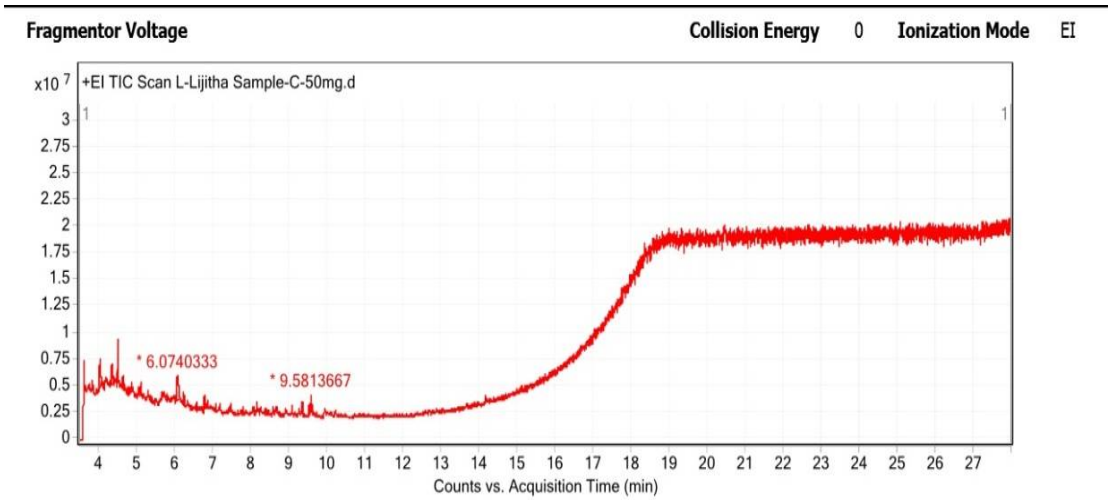
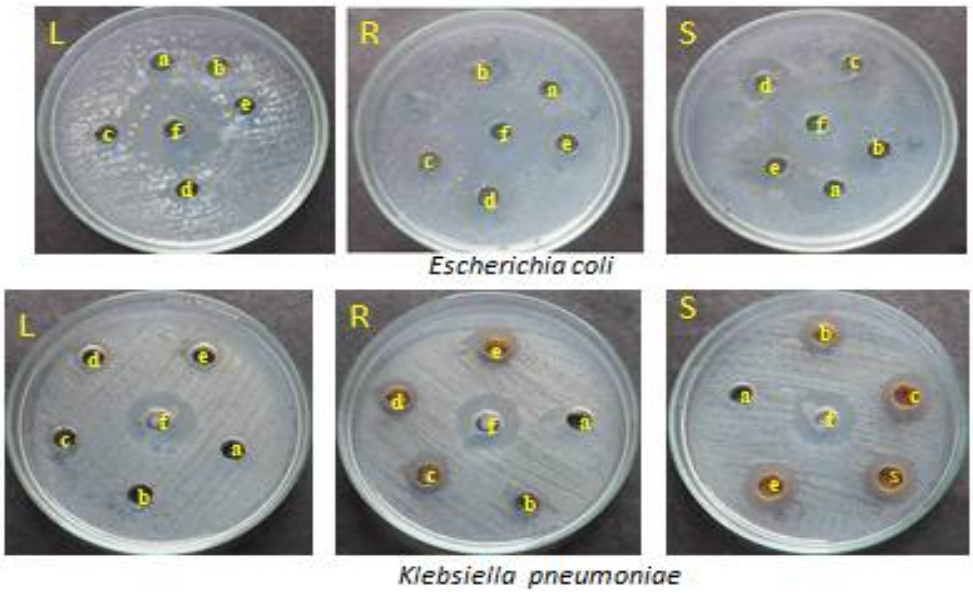


Figure 3: Chromatogram of the aqueous stem extract of *Solanum indicum*



a: 0µg/well; b: 25µg/well; c:50µg/well; d:75µg/well; e:100µg/well; f: 30µg/well (Azithromycin – Positive control)

Figure 4: Antibacterial activity of leaf (L), root (R) and stem (S) extracts) of *Solanum indicum* against *Escherichia coli* and *Klebsiella pneumonia*

DISCUSSION

Plants have always been focused as functional sources for antioxidants and antimicrobial agents to promote health and treat various diseases. The difference in the yield obtained from leaves, root, and stem is due to the variation in the presence of biological components in different parts of *Solanum indicum*. In the present study, the preliminary qualitative analysis of the various parts (leaves, root, and stem) of *Solanum indicum* confirmed the presence of secondary metabolites like phenolic compounds, saponins, alkaloids, glycosides, flavonoids, and terpenoids. The major secondary metabolites, phenolic compounds (tannins, flavonoids, terpenes etc.,) and nitrogen compounds (alkaloids, amino acids etc) are potent antioxidants, acts by removing the free radicals, inhibiting the enzymes responsible for the free radical formation, enhancing the concentration or activity of endogenous antioxidants and by chelating the metal ions [36]. The alkaloids, saponins, terpenoids, carbohydrates, and flavonoids in plant sources serve as potent antimicrobial agents [37]. The synergistic biological activities of these secondary metabolites may be the reason for the therapeutic potential of *Solanum indicum*.

In the present study, the amount of phenols and flavonoids was more in the aqueous root extract when compared with the leaves and stem portions of *Solanum indicum*. Phenolic compounds have one aromatic ring with one or more hydroxyl groups whereas flavonoids have two benzene rings separated by a propane unit. The phenolics have attributed to the biological activities from the level of an antioxidant to anticancer potential [38]. The polyphenolic nature of flavonoids is rendered with activities like mitochondrial adhesion inhibition, antiulcer, antiarthritic, anticancer, antiangiogenic, protein kinase inhibition, antimicrobial, etc. [39]. Our findings were in congruence with the findings in *Salvia officinalis* where a high concentration of flavonoids and phenolic secondary metabolites were found in the aqueous extract [40].

The result shows that the amount of tannins is more in the aqueous root extract of *Solanum indicum*. Tannins could effectively interact with hydroxyl radicals, quench free radicals, donate hydrogen atoms, and form complexes with protein to scavenge the free radicals [41]. Tannins were known to exhibit bactericidal activity either by damaging the bacterial membrane or by penetrating through the cell wall and interferes with bacterial metabolism or forming complexes with enzymes/substrates or by chelating the ions essential for the survival of bacteria [42] and / inhibits the attachment of bacteria [43]. Tannins bind to the receptor of the cell and thus inhibit the binding of the viruses and also prevent the attachment of proteins essential for viral metabolism [43]. From the observations, it appears that Vitamin C is predominantly present in root extract followed by leaves and stem of *Solanum indicum*. Ascorbic acid primarily present in plants acts as good reductones by donating hydrogen, breaking the radical chain, and reacts with peroxides [44, 32]. The results obtained in the present study confirm the antioxidant, antibacterial and antiviral properties manifested by *Solanum indicum* were due to the secondary metabolites present in it. Further, the results also proved that not only the root portion but also leaves and stem parts of *Solanum indicum* would serve as a potent source of antioxidants.

In this study, the aqueous root extract of *Solanum indicum* was found to be most potent to scavenge DPPH radicals. The activity noted may be due to the appreciable amount of phenols, flavonoids, tannins, and vitamin C reported in the root extract of *Solanum indicum*. The phenolic compounds exhibit their antioxidant property by quenching the free radicals or chelate the metal ions or neutralize them by transferring the electrons or hydrogen. Thus the presence of these secondary metabolites in *Solanum indicum* could act as antioxidants to scavenge any type of reactive oxygen species and prevent oxidative damage. Dieu-Hien et al. [45] have observed that the methanolic extract of *Severinia buxifolia* was more powerful than ascorbic acid in scavenging DPPH due to its phytoconstituents.

Lysosomal membranes were ruptured during inflammation and the release of lytic enzymes further complicates the inflammatory process leading to inflammatory disorders. Since RBC and lysosomal membrane are similar in structure, prevention of hemolysis was taken as an index to assess the anti-inflammatory potential. In this study, *Solanum indicum* extracts demonstrated potent anti-inflammatory activity by stabilizing the RBC membrane. Erythrocytes are attacked by the free radicals causing membrane disorganization and its lysis [46]. Flavonoids and phenols possess the ability to stabilize the membrane of mast cells [47, 48]. In this study, prevention of lysis may be due to the antioxidant, anti-inflammatory, and membrane stabilizing potential contributed by the phenols, flavonoids, tannins, and vitamin C in the extracts of *Solanum indicum*. In agreement with the current findings, Dieu-Hien et al. [45] has found *Severinia buxifolia* as the source for potent anti-inflammatory agents due to the presence of above said compounds. The overall findings suggest that the phytoconstituents in *Solanum indicum* could stabilize the membrane of lysosomes and thus reduce the course of the inflammatory disease and prevents the harmful effects of the released mediators.

The phytoconstituents in *Solanum indicum* are responsible for their therapeutic potential. Most of the compounds possess varied biological activities (Table 8). Among them, 3 compounds – paromomycin,

tetra acetyl-d-xylonic nitrile and acetamide, N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl]- are common in all three parts of *Solanum indicum*. The compound paromomycin has antimicrobial activity [49]. Tetraacetyl-d-xylonic nitrile has anti-oxidative activities and anti-viral effects [50] and Mudiganti et al. [51] too has reported that it could act as beta-hydroxysteroid dehydrogenase inhibitor, alcohol dehydrogenase inhibitor, anticancer agent, antidote, CNS depressant, coronary dilator, and decongestant. Acetamide, N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl]- was known for its potent anti-inflammatory properties [52].

It was found that the compound catechol is present both in leaves and root of *Solanum indicum* which possess antioxidant, antiplatelet, and antimicrobial potential [53, 54) and it serves as estrogen backbone and stimulant [51]. 5, 8, 11, 14-eicosatetraenoic acid, methyl ester,(all-z)- with antifungal, antibacterial, antitumor and cytotoxic effects [55] was present in leaf and stem parts of *Solanum indicum*. Another compound bicylo(3.3.1) nonan-9-one, 2-(1-pyrrolidinyl)- (alkaloid) present in the root and stem parts of *Solanum indicum* is widely used in the treatment of stomach pain [56]. Some compounds were present only in leaves of *Solanum indicum* like Pyrrolizin-1,7-dione-6-carboxylic acid, methyl (ester) with anti-viral and anti-tumor activity [50], folic acid, the vitamin is essential for hemoglobin synthesis and maintenance of mental and emotional health [57], Phenol, 3-methyl-5-(1-methyl ethyl)-, methylcarbamate serves as oligosaccharide provider, catechol-O-methyl transferase inhibitor, methyl donor, methyl guanidine inhibitor [54]. No activity has been reported for 2-pyrrolidin-1-yl-bicyclo [3.3.1] nonan-9-ol and adenosine-3-phosphoric acid compounds in aqueous leaf extract of *Solanum indicum*.

The aqueous root extract of *Solanum indicum* posses some unique compounds like 4H-pyran-4-one, 2,3, dihydro-3,5-dihydroxy-6-methyl-, Ethanone, 1-(2-hydroxy-5-methylphenyl)-, 1H-6-purinone,6,7-dihydro-2-amino-7[3,5,-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-2-pyranyl, α-D-Glucopyranoside, O-α-D-glucopyranosyl-(1.fwdarw)-β-D-fructofuranosyl and Columbin which is not present in leaf and stem extracts. Surprisingly out of these, α-D-Glucopyranoside, O- α-D-glucopyranosyl-(1.fwdarw)-β-D-fructofuranosyl was found to be the predominant compound with peak area (23.89%) was reported to have anti-diabetic activity [50]. The next important compound 4H-pyran-4-one, 2,3, dihydro-3,5-dihydroxy-6-methyl (18.54%) was found to be an insecticidal agent [58] and also with antimicrobial and anti-inflammatory activities [59]. No activity has been reported for Ethanone, 1H-6-purinone,6,7-dihydro-2-amino-7[3,5,-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-2-pyranyl and Columbin.

Results of aqueous stem extract of *Solanum indicum* have revealed the presence of 9 compounds in which 4 compounds were unique. Cyclohexanone has antibacterial activity [60]. 1-Heptatriacotanol works as an anti-microbial agent [61] and as an enzyme inhibitor and also has anti-hypercholesterolemic effects [62]. Oleic acid is an acidifier, arachidonic acid inhibitor, inhibits production of uric acid and increases the aromatic amino acid decarboxylase activity [51]. For Cyclopropanebutanoic acid- 2[[2-[[2-[(2-pentylcyclopropyl) methyl]cyclopropyl]methyl]cyclopropyl]methyl]-,methyl ester no activity is known.

Thus the results obtained from GC-MS analysis have provided much valuable information about the phytocompounds in root, stem, and leaf parts of *Solanum indicum*. As only the root part of *Solanum indicum* is used in traditional medicine, the obtained results have disclosed the presence of potentially active compounds with antioxidant, anti-inflammatory, antitumor, anti hypercholesterolemic, antimicrobial, antibacterial, antifungal, and antifungal potential in leaves and stem. Thus this will open a new avenue to explore the potential of leaves and stem parts of *Solanum indicum*.

In the current study, the aqueous extract of stem and root parts of *Solanum indicum* is more active than the leaf extract against both *Escherichia coli* and *Klebsiella pneumonia*. This difference might be due to the variation in the phenols and flavonoid content in root and stem parts than leaves of *Solanum indicum* and also due to the synergistic effect of various phytoconstituents in *Solanum indicum*. Similar results were reported by Sanchez et al. [63], where better antibacterial and anti-biofilm activity was observed in ethanolic extract of *Cytinus* than water extract. The study of GC-MS revealed the presence of paromomycin compound in all the parts (leaves, root, and stem) of *Solanum indicum* with reported antibacterial/antimicrobial properties in earlier studies. The better bactericidal property of the aqueous stem extract of *Solanum indicum* might be due to the presence of compounds paromomycin (30.5%), cyclohexanone (8.58%), 1-Heptatriacotanol (5.47%), and 5,8,11,14-eicosatetraenoic acid, methyl ester,(all-z) (4.59%) with antibacterial potential in predominant amount when compared with root and leaf extracts. Thus the results obtained provide a promising platform to utilize the stem and root parts of *Solanum indicum* for the development of antimicrobial products to fight against antibiotic-resistant microbes especially *Escherichia coli* and *Klebsiella pneumonia*, the most common pathogens of urinary tract infections (UTI).

CONCLUSION

The present study reports that *Solanum indicum* is a good source of natural antioxidants. The aqueous extract of *Solanum indicum* roots showed the highest amount of phenols, tannins, flavonoids, and Vitamin C and exhibited better DPPH scavenging potential and anti-inflammatory potential than the leaves and stem. Better antimicrobial activity was evidenced in the stem part of *Solanum indicum*. Twenty-one compounds were identified from all the parts (leaves, root, and stem) of *Solanum indicum* by GC-MS analysis. The biological activities of these compounds were responsible for their therapeutic potential. Though all parts are used in folk medicine and prescribed by traditional healers, only roots of *Solanum indicum* find a place in Ayurvedic medicine. The results of the present study have provided the scientific validation for indigenous knowledge of traditional healers on the use of leaves and stem parts of *Solanum indicum* for treatment. The metabolites identified in the leaves and stem parts of *Solanum indicum* will open a new possibility to explore their pharmacological activity.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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