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PHYTOCHEMICAL, ANTIOXIDANT AND ANTIMICROBIAL ANALYSIS OF *SOLANUM LYCOPERSICUM ESCULENTUM* LEAF EXTRACT

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ABSTRACT

Background: *Solanum lycopersicum esculentum (Tomato) leaves are considered as poisonous material globally, because of the tomatine and solanine toxin. Despite the fact of its poisonous, the plants possess many phytochemicals, antioxidants and antimicrobial activities.* ***Objective:*** *the present study was aimed to in vitor phytochemical, antioxidant and antimicrobial analysis of Solanum lycopersicum esculentum leaves.* ***Methods:*** *Solanum lycopersicum esculentum leaves were subjected to soxhlet extraction apparatus with hydroethonol solvent.* ***Results:*** *Phytochemical analysis of the crude extract proves the presence of alkaloids, flavonoids, phenols, tannins, saponins. Hydroethanolic leaf extract was tested on gram-positive Staphylococcus aureus, streptococcus pyogens and gram-negative E. coli, Pseudomonas aerogenous, enterococcus fecalls, Bacillus subtillis subsp spizizeinii.* ***Conclusion:*** *Zone of inhibition proves that the solanum lycopersicum esculentum hydroethanolic leaf extract possess antimicrobial property.*

KEY WORDS

antioxidants, antimicrobial, Hydroethanolic, Phytochemical, and Solanum lycopersicum esculentum.

INTRODUCTION

In the earth there are about 3, 90,000 vascular plants are identified. Among them, many serve as an edible and few are used as an herb in drugs and medicines. Other than compatible plants many noxious plants are also noted worldwide. Indigenous varieties of plants were used in rural or countryside as a medication. So, known from ancient days all the plants are said to have much activity in nature against critical issues arising both in humans and plants caused by the pathogenic agents such as microorganisms (bacteria, virus, fungi, protozoa). These activities are prevented by the use of several methods and applications nowadays [1].

But claiming ancient medicines with added changes can bring many unimaginable dynamic curative treatments. Phytochemicals present in plants are responsible for their medicinal activity [2]. Solanum *Lycopersicum esculentum*, commonly known as a tomato is a good nutrient rich vegetable, having vast effect against various disease-causing agents [3]. Tomato is the major constituent for many food dishes. The plant belongs to the nightshade family, solanaceae, and has the native of south west America. The tomato leaves contain volatile components such as 2- carene which is used as a chemical intermediate and in perfume production [4]. And also the tomato plant is known as a good source of phenolic compounds, pigments [5]. Some of the studies revealed the toxicity of the Solanum *Lycopersicum esculentum* leaves by the presence of solanine and tomatidine toxins as secondary metabolites [6]. The tomato leaf volatiles (TLV) also act as an antifungal agent against plant pathogenic fungi [7]. Based on the above information the present study was subjected to antioxidant, antibacterial activity of Solanum *Lycopersicum esculentum* leaf extracts.

MATERIALS AND METHODS

Collection of plant

Solanum lycopersicum esculentum leaves were collected in the month of May from various place of Coimbatore, collected leaves were dried at room temperature for 7-10 days. Collected plant material was identified and authenticated (BSI/SRC/5/23/2018/Tech./1508) at Botanical Survey of India (South Zone) Coimbatore and the voucher specimen was deposited in the department of Microbiology, Dr. N.G.P.Arts and Science College, Coimbatore.

Solvent extraction

The dried leaves were pulverized to fine quantity for further procedures. 50 gram of leaf powder was weighed, added with 150 ml (50 ml water+100ml ethanol) of hydroethanol. The sample was extracted using the Soxhlet apparatus. Upon the completion of the process 50ml of the extract was obtained.

Phytochemical analysis

The hydroethanolic leaf extract of *Solanum lycopersicum esculentum* was subjected for the phytochemical analysis [8].

Test for alkaloids

About 2ml of the leaf extract was added with 2-3 drops of Wagner's reagent. Brown colour appearance will take place in the presence of alkaloids.

Test for flavonoids

Along with a pinch of leaf powder 10 ml of distilled water was added. Followingly 3-5 drops of dilute ammonia were added. The formation of yellow color upon the addition of con H_2So_4 indicated the presence of flavonoids in the leaf extract.

Amino acids test

To the 3ml of leaf extract 2-5 drops of ninhydrin reagent was added. The formation of blue colour indicates the presence of amino acids in the leaf extract.

Test for tannins

To the 2ml of leaf extract 2-3 drops of ferric chloride (5% $FeCl_3$). The appearances of dark blue green colour indicate the presence of tannins.

Test for reducing sugar

Few drops of Felling solution were added to the leaf extract and kept at 40°C in water bath. Formation of red precipitate indicates the presence of reducing sugar.

Test for saponins

To 2ml of a leaf extract 5ml of distilled water was added. Formation of persistent foam indicates the presence of saponins.

Test for phenols

To 1ml of leaf extract 2ml of distilled water was added. Along with that 2-3 drops of 10%, aqueous $FeCl_3$ was also added. Formation of green precipitate indicates the presence of phenols.

Test for steroids

About 1ml of leaf extract was added with 2ml of acetic anhydride. With this 3-5 drops of chloroform and 2drops of Con.H_2SO_4 was added.

Test for phytosterols

To 2-3 drops of chloroform was added to 2ml of leaf extract. The formation of dark pink color upon the addition of anhydride and Con.H_2SO_4 indicate the presence of phytosterols.

Test for proteins

Few drops of ninhydrin was added to 2ml of leaf extract. The formation of blue color indicates presence of proteins.

***In vitro* antioxidant assay**

DPPH Assay

The free radical scavenging activity of hydroethanolic leaf extract of *Solanum lycopersicum esculentum* was measured by using DPPH (2, 2-diphenyl-1-picrylhydrazyl). The scavenging activity for DPPH free radicals was measured according to the procedure [11]. About 1ml of 0.1mM DPPH solution (<1.00 at 517 nm in spectrophotometer) was mixed 5µl, 10µl, 15µl, 20µl, 25µl of *Solanum lycopersicum esculentum* hydroethanolic leaf extract. Flowingly equal volume of methanol was added and kept at dark room temperature for 30 minutes. Standard ascorbic acid and absorbance adjusted DPPH solution was used as positive and negative controls in the assay. Spectrophotometric absorbance of the mixtures was measured at 517 nm. The percentage inhibition of DPPH radical quenching by the sample as well as standard was calculated as follows:

$$\text{DPPH Scavenging in \%} = \frac{\text{OD of sample - OD of control}}{\text{OD of control}} \times 100$$

FRAP Assay

The (ferric reducing ability of plasma) antioxidant capacity of plant leaf extracts were estimated according to the procedure [12]. 95 µl, 90µl, 85 µl, 80 µl, 75 µl, distilled water was added to 5µl, 10µl, 15µl, 20µl, 25µl hydroethonolic leaf extract of *Solanu lycopersicum esculentum,* and incubated at room temperature for 30 minutes. After incubation the solution were read at 593nm in spectrophotometer. The optical density of the leaf extract was compared with control and their values are calculated using the formula.

Scavenging activity in % = control- test / control X 100

Nitric oxide Reductase assay

NOR scavenging activity of plant leaf extract was examined by the standard procedure [13, 14]. 400 µl of 100mM sodium nitro prusside and 100 µl of PBS **(pH 7.4)** was added to 0.5, 1.0, 1.5, 2.0, 2.5 µl of *Solanu lycopersicum esculentum* hydroethonolic leaf extract. The reaction mixture was kept into 25°C for 150 minutes. After incubation 0.5ml of Gris reagent was added and kept in room temperature for 30 minutes. The optical density of the leaf extract was compared with control all the reactions were performed in triplicates and their percentage inhibition was calculated by the following formula:

Scavenging activity in % = (($A_{control\ I}$ - B_{sample}) / $A_{controll}$) ×100

Antimicrobial activity

The antibacterial activity of crude hydroethanolic leaf extract was determined by Well Diffusion method [15]. Hydroethanolic leaf extract of *solanum Lycopersicum esculentum* was tested against the clinically important strains. The chosen organisms are swabbed on Muller Hinton agar and left for 10 minutes with the aid of cork porer, 6mm diameter of wells was created in the plates. A 0.5ml of each concentration of *Solanum lycopersicum esculatum* hydroethanolic leaf extract was added in the wells. A negative control was 0.01ml of the extracting solvent. The plates were allowed on the bench for 30 minutes for pre diffusion of the extract to occur. The treated Petri plates were incubated at 37°C for 24 h. Each experiment was carried out in triplicate.

Statistical Analysis

Experiments were carried out in triplicate and the results are expressed as mean values with standard deviation.

RESULTS AND DISCUSSION

Phytochemical analysis

Phytochemical screening of Hydroethanolic leaf extract *Solanum lycopersicum esculentum* revealed the presence of alkaloids, flavonoids and phenols in high level, whereas saponin, tannin and sterols present in moderate level (*Table3.1*).

The presences of various phytochemical are believed to be responsible for the antibacterial effects [16]. Flavonoid derivatives were reported to be effective antimicrobial substances against different microorganisms. Their mode of activity may be due to their ability to complex with extracellular and soluble proteins as well as to complex with bacterial cell wall. The flavonoids being more lipophilic may also disrupt microbial membranes. In addition to being effective against bacteria, these compounds exhibit inhibitory effects against viruses and parasites [17]. In the present study, the Hydroethanolic leaf extracts *Solanum lycopersicum esculentum* revealed the presence of alkaloids, flavonoids and phenols in high level which may responsible for the antibacterial and antioxidant activity.

***In vitro* antioxidant assay**

Free radical scavenging activity determined by DPPH, FRAP, and NOR methods in the smaller concentrations showed superior activity were mentioned in *table 3.2.1, 3.2.2, 3.2.3*.

The scavenging capability of DPPH was determined by the decrease in its absorbance at 517 nm as well as by the degree of colour change from deep violet to yellow. Both ascorbic acid and Hydroethanolic leaf extract *Solanum lycopersicum esculentum* showed dose dependent activity. In the present study the percentage radical scavenging property of the Hydroethanolic leaf extract *Solanum lycopersicum esculentum* and ascorbic acid was 66.82% and 78.27% at 25 µg/mL respectively (*Table 3.2.1*).

The data in the literature related to DPPH radical scavenging activity of tomatoes are limited; however, [18] reported the DPPH radical scavenging activity of tomato cultivars between 97 and 98%. In this study, the inhibition effect of tomatoes on DPPH radical was found 75.88% in the concentration of 25µg/ml, which was comparably equal to ascorbic acid showed 78.27% in the higher concentration (25µg/ml).

The Ferric Reducing Ability of Plasma (FRAP) is presented as a novel method for assessing "antioxidant power". FRAP values are obtained by comparing the absorbance change at 593 nm in test reaction mixtures with those containing ferrous ions in known concentration. Hydroethanolic leaf extract of *Solanum lycopersicum esculentum* showed significant activity at the concentration of 25µg/ml were mentioned in *table 3.2.2*.

Nitric Oxide Reductase assay of Hydroethanolic leaf extract *Solanum lycopersicum esculentum* was carried out and the activity of plant extract was mentioned in *table 3.2.3*. Hydroethanolic extract showed significant activity at the concentration of 25µg/ml which showed comparable activity to standard ascorbic acid.

Antibacterial assay

Medicinal plants are among the richest bio-resources of the drugs currently used to treat bacterial and other infections [19, 20]. In the present study, the crude Hydroethanolic leaf extract of *Solanum lycopersicum esculentum* showed significant activity against various clinical pathogens and the zone of inhibition were mentioned in table 3.3. The crude extract showed highest zone of inhibition 35.33±0.24mm at the highest concentration 100 µg/ml against Bacillus spp, followed by Pseudomonas (30.33±0.47) spp and Enterococcus (30.33±14), whereas showed moderate activity against Staphylococcus spp. and Streptococcus spp.

The present study reveals the Hydroethanolic leaf extract of *Solanum lycopersicum esculentum* exhibited highest zone of inhibition against gram negative pathogens than gran positive pathogens. These findings are in agreement with a previous report [21], which showed that Gram-negative strains are in general more resistant than Gram-positive ones in well diffusion tests. This higher resistance could be due to the external lipopolysaccharide wall that surrounds the peptidoglycan cell wall of the formers [22] or to the occurrence of membrane accumulation mechanisms. Our findings displayed, *Solanum lycopersicum esculentum* significantly reduced the growth of gram negative pathogens.

Table 3.1. Phytochemical analysis of hydroethanolic leaf extract of *Solanum lycopersicum esculentum*

S.NO	CONSTITUENTS	HELE of *S. lycopersicum esculentum*
1	Alkaloids	+++
2	flavonoids	++
3	Amino acids	-
4	Reducing sugars	-
5	Saponins	+
6	Phenols	++
7	Tannins	+
8	Steroids	+
9	Phytosterols	-
10	Proteins	-

+++ = Abundantly present, ++ = Moderately present, + = present, - = Absent, HELE = hydroethanolic leaf extract.

Table 3.2.1. DPPH free radical scavenging assay of *Solanum lycopersicum esculentum*

S.No	Concentration of sample (µg/ml)	DPPH ACTIVITY (%)	Standard (ascorbic acid) (%)
1.	5	17.63	30.66
2.	10	44.11	45.11
3.	15	59.57	58.65
4.	20	68.79	65.78
5.	25	75.88	78.27

Table 3.2.2. FRAP assay of Hydroethanolic leaf extract *Solanum lycopersicum esculentum*

S.No	Concentration (µg/ml)	FRAP ACTIVITY (%)	Standard (ascorbic acid) \ (%)
1.	5	47.01	40.05
2.	10	52.98	50.10
3.	15	58.27	55.01
4.	20	71.52	68.05
5.	25	81.45	78.50

Table 3.2.3. Nitric Oxide Reductase assay of Hydroethanolic leaf extract *Solanum lycopersicum esculentum*

S.No	Concentration (µg/ml)	NOR ACTIVITY (%)	Standard (ascorbic acid) (%)
1.	5	39.04	42.01
2.	10	43.08	48.20
3.	15	59.00	61.01
4.	20	64.00	68.00
5.	25	87.00	84.20

Table 3.3. Antibacterial activity of Hydroethanolic leaf extracts *Solanum lycopersicum esculentum*

	Concentration (µg/ml) / Zone of inhibition in mm					
Name of the organisms	**20**	**40**	**60**	**80**	**100**	**Chloramphenicol**
Pseudomonas spp.	0	23.66±0.47	26.33±0.47	28.66±0.47	30.33±0.47	35.66±0.47
E.coli	0	24.33±0.47	26.33±0.94	29.33±0.47	29.33±.047	32.33±0.47
Staphylococcus spp.	17.66±0.47	22±0.82	27.33±0.47	28.33±0.94	28.33±0.47	29.33±0.22
Streptococcus spp.	16.66±0.45	17.33±0.42	19.66±0.41	20.66±0.39	24.65±0.32	27.61±0.12
Bacillus	0	28.45±0.23	29.45±0.25	31.65±0.25	35.33±0.24	26.34±0.42
Enterococcus	14.62±0.24	17.26±0.32	21.26±0.95	25.36±0.19	30.33±14	32.45±0.24

CONCLUSION

The present study deals with various parameters phytochemical, antioxidant, and antimicrobial activity and concluded that the Hydroethanolic leaf extract of *Solanum lycopersicum esculentum* exhibited varying level of phytoconstituents, antioxidant and antibacterial activity. The present finding provided basic information of the plant's potential and leads to further identification of bioactive compound.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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