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Antimicrobial and antioxidant activities of salt stress callus of Brinjal *(Solanum melongena* L.)

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Abstract— *Ethanolic and methanolic salt stress callus extracts of Solanum melongena L. were tested for in vitro antimicrobial and free radical scavenging assayssuch as DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS$^+$(2,2'Azinobis (3-ethyl benzo-thizoline-6-sulfonic acid) . In both the extracts the zone of inhibition is higher in Escherichia coli, Klebsiella pneumonia, Staphylococcus aureusand Streptococcus pyogenesat 90 µl concentration against the control. The antifungal activity of these extracts also the zone of inhibition is higher at 90 µl concentration against the control. The DPPH activity of different concentration of solvent extracts (1 mg/ml to 5 mg/ml) along with standard ascorbic acid among the five different concentration (50 µg/ml to 250 µg/ml) of extracts tested, the higher percentage of inhibition was observed in 250 µg/ml of methanol extract followed by ethanolic extract against the standard ascorbic acid. In ABTS$^+$ activity the absorbance was increased with the increasing concentrations of both methanolic and ethanolic callus extracts.*

Keywords—Antioxidant, , Inhibition, In vitro, Stress tolerant callus, Solanum melongena.

I. INTRODUCTION

Plants have been an important source of medicine, mainly on traditional remedies for thousands of years [1]. The bioactive substances present in plants have wide range of biological functions including antioxidant and antimicrobial activities [2,3]. Environmental stresses strongly influence plant growth and development. Through biotechnology tools, the production of virus free plants, salinity tolerance, herbicide resistance, frost resistant is passible [4]. These stress agents influence on biosynthesis of secondary metabolites and resulting in considerable fluctuations in quality and quantity. The composition of secondary product may vary within the same plant. Generally, the efficacy of the plant depend on the combined effect of plant metabolites rather than the few fractions separated from the plant. This leads to select a plant *S. melongena* L. for the changes in biochemical activities under salt stress.

Brinjal or Eggplant (*Solanum melongena* L.) is an important vegetable crop belong to the family Solanaceae. The family contain 75 genera and around 2000 species. In India, it is represented by 21 genera and 70 species. Brinjal is an herbaceous perennial plant but cultivated as annual. In popular medicine, brinjal is indicated for the treatment of several diseases, including diabetes, arthritis, asthma and bronchitis. In addition, that brinjal extracts have a significant effect in reducing blood and liver cholesterol in humans [5,6]and adult rats [7]. Nasunin, a major component of anthocyanin pigment of brinjal, has been shown to inhibit lipid peroxidation [8]. Free radical scavenging and iron chelating activities of nasunin were demonstrated by electron spin resonance [9]. Furthermore, anti-mutagenic activity of pheophytin components from brinjal fruit extracts acting against several chemical mutagens was reported [10]. The unripe fruit of brinjal is primarily used as a cooking vegetable for the various dishes in different regions of the World. It has much potential as raw material in pickle making and dehydration industries [5,6].

Understanding the importance of salt tolerance in crop plants such as brinjal, the present work is to focus on the production of salinity tolerance brinjal callus through *in vitro* culture technology and to analyze antioxidant and antimicrobial activity of ethanol and methanol extracts of *in vitro* salt callus.

II. MATERIALS AND METHODS

2.1 Preparation of salt callus extracts

The leaf derived 40 days old 20 gram of powdered brinjal salt callus was successively extracted using 50 ml of ethanol and methanol by using the Soxhlet extractor for 8-10 hrs [11]. The extract was filtered through Whatmann No.1 filter paper to remove all undissolved matter including cellular materials and other constitutions that are insoluble in the extraction solvent. The respective extracts were concentrated in vacuum (Rota vapor) and

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the residues from the ethanol and methanol extracts were weighed and stored in sealed vials in a freezer until tested.

2.2 Antimicrobial activity

2.2.1 Test microorganisms

The test organisms used were clinical isolates *viz., Streptococcus pyogenes, Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumoniae*and the human fungal pathogens like *Candida albicans* and *Trichoderma viride*, which were obtained from Department of Microbiology, Hindusthan College of Arts and Science Coimbatore. The bacterial and the fungal cultures were maintained on nutrient agar medium at 37˚C and potato dextrose agar (PDA) medium at 28˚ C respectively.

2.2.2 Preparation of Inoculum

The gram positive bacteria *Streptococcus pyogenes*, *Staphylococcus aureus* and gram negative bacteria *E. coli* and *Klebsiella pneumoniae* were pre-cultured in nutrient broth overnight in a rotary shaker at 37° C, centrifuged at 10,000 rpm for 5 min, pellet was suspended in double distilled water and the cell density was standardized spectrophotometrically (A_{610} nm). The fungal inoculums *Candida albicans*, *Trichoderma viride* were prepared from 5 to 10 day old culture grown on Potato dextrose agar medium. The Petri dishes were flooded with 8 to 10 ml of distilled water and the conidia were scraped using sterile spatula. The spore density of each fungus was adjusted with spectrophotometer (A_{595}nm) to obtain a final concentration of approximately 10^5 spores/ml.

2.2.3 Antibacterial Activity [12]

The *in vitro* salt callus extracts of *S. melongena* were tested by the well diffusion method. Different concentration of the extracts (30, 60, and 90 µg/ml) was prepared by reconstituting with ethanol and methanol. The test microorganisms were seeded into respective medium by spread plate method 10 µl (10 cells/ml) with the 24h cultures of bacteria growth in nutrient broth. After solidification the filter paper wells (5 mm in diameter) impregnated with the extracts were placed on test organism-seeded plates. Streptomycin (10 µg) used as standard for antibacterial test. The antibacterial assay plates were incubated at 37°C for 24 hrs. The diameters of the inhibition zones were measured in mm.

2.2.4 Antifungal Activity [13]

The antifungal activity of ethanol and methanol *in vitro* salt callus were tested by well diffusion method. The potato dextrose agar plates were inoculated with each fungal culture (10 days old) by point inoculation. The filter paper wells (5 mm in diameter) impregnated with 20, 40 and 60µg/ml concentrations of the extracts were placed on test organism-seeded plates. Streptocycline (10 µg well 1) used as positive control. The activity was determined after 72 hrs of incubation at 28°C. The diameters of the inhibition zones were measured in mm.

2.3 Anti-oxidant activity

2.3.1 DPPH Radical Scavenging Activity

DPPH radical is scavenged by antioxidants through the donation of a proton forming the reduced DPPH. The colour change from purple to yellow after reduction can be quantified by its decrease in absorbance at wavelength 517 nm [14]. Various concentrations of ethanol and methanol extracts of the sample (0.52.5 mg/ml) were mixed with 1.0 ml of ethanolic and methanolic solution containing DPPH radicals, resulting in the final concentration of DPPH being 0.2 mM. The mixture were shaken vigorously and left to stand for 30 min, and the absorbance was measured at 517 nm. Ascorbic acid was used as control. The percentage of inhibition in DPPH radical scavenging activity was calculated as follows

$$\% \text{ Inhibition} = A_0 - A_1/A_0 \text{ X } 100.$$

2.3.2 $ABTS^+$ Radical scavenging activity

$ABTS^+$ decolourisation assay involves the generation of the $ABTS^+$ chromophore by the oxidation of $ABTS^+$ with potassium persulphate. It is applicable for both hydrophilic and lipophilic compounds. The scavenging activity of the leaf extracts on $ABTS^+$ radical cation was measured at 734 nm[15].

$ABTS^+$ solution: Equal volume of 7 mM of $ABTS^+$ was mixed with 2.45 mM potassium persulphate and the mixture was allowed to stand in the dark at room temperature for 12-16 hours before use. $ABTS^+$ solution was diluted to an absorbance of 0.7 ± 0.05 with ethanol and methanol 734 nm. The reaction was initiated by the addition of 1.0 ml of diluted $ABTS^+$ to 10 µl of different concentrations (50 - 250 µg / ml) of leaf extract and also to 10 µl of ethanol and methanol as control. Ascorbic acid was used as positive control. The absorbance was read at 734 nm after 6 minutes and the percentage inhibitions were calculated. The inhibition was calculated according to the equation,

$$I = A_0 - A_1/A_0 \text{ x } 100,$$

Where, A_0 is absorbance of control reaction, A_1 is absorbance of test compound.

III. RESULTS

3.1 Antimicrobial activity

The antimicrobial activity of ethanolic and methanolic extracts of *S. melongena* salt callus against various microbial strains with respect to various concentrations (30 – 90µg/ml) were presented in the table 1. In *S. melongena* salt callus ethanolic extract, the zone of inhibition of test concentrations were compared with standard concentration of control (Streptocycline 10 µg/ml). Plate. 1A, B, C, and D shows significant result of different concentration of extract and the control. Among the four different bacteria used (*E.coli,K. pneumonia,S.*

aureus, S. pyogenes) in the case of *E.coli* the zone of inhibition is higher (13.60±0.20 mm) in 90 µg/ml concentration (Plate. 1A) against the control (08.50±0.17 mm10µg/ml), followed by 60 µg/ml concentration (09.66±0.17 mm). In the case of *S. aureus* the zone of inhibition is higher in 90 µg/ml concentration (12.70±0.17 mm) against its control (10mm/ 10µg/ml) (Plate. 1C) followed by 60 µg/ml concentration (07.66±0.14 mm). In *K. pneumonia* also the maximum inhibition zone (11.83±0.12 mm) was observed in 90 µg/ml concentration followed by 60 µg/ml (06.56±0.26 mm) (Plate. 1B) against the control (07.50±0.23 mm). At 90 µg/ml of concentration the zone of inhibition is higher (09.56±0.14 mm) followed by 60 µg/ml concentration (06.56±0.20 mm) in *S. pyogenes* against control (10.53±0.20 mm) (Plate. 1D, Table 1).

In methanolic extract among the four different bacteria used, in the case of *S. aureus* the zone of inhibition is higher in 90 µg/ml concentration (12.63±0.14 mm) against its control (10mm/ 10µg/ml) (Plate. 2C) followed by 60 µg/ml concentration (07.50±0.20 mm). In the case of *E.coli* the zone of inhibition is higher (12.50±0.17 mm) in 90 µg/ml concentration (Plate. 2A) against the control (8.73±0.08 mm1 µg/ml), followed by 60 µg/ml concentration (8.46±0.14 mm). In *K. pneumonia* also the maximum inhibition zone (12.13±0.26 mm) was observed in 90 µg/ml concentration followed by 60 µg/ml (8.53±0.12 mm) (Plate. 2B) against the control (8.80±0.11 mm) respectively. At 90 µg/ml of concentration the zone of inhibition is higher (11.23±0.23 mm) followed by (8.63±0.17 mm) at 60 µg/ml in *S. pyogenes* against control (10.63±0.14 mm) (Plate. 2D, Table 1).

The human fungal pathogens like *Candida albicans* and *Trichoderma viride*, the zone of inhibition was observed in ethanolic extracts of salt callus compared with standard drug and presented in Table 1. Among the two different fungal used, in the case of *C. albicans* the zone of inhibition is higher (8.63±0.21 mm) in 90 µg/ml concentration (Plate. 1E) against the control (8.63±0.12 mm1 µg/ml), followed by 60 µg/ml concentration (5.66±0.24 mm). In the case *T. viride* the zone of inhibition is higher in 90 µg/ml concentration (6.80±0.15 mm) against its control (8.70±0.15 mm/ 10µg/ml) (Plate. 1F) followed by 60 µg/ml concentration (5.56±0.08 mm).

In methanolic extracts *C. albicans* the zone of inhibition is higher (8.73±0.21 mm) in 90 µg/ml concentration (Plate.2E, Table 1) against the control (8.43±0.23 mm10µg/ml), followed by 60 µg/ml concentration (5.56±0.26 mm). In the case *T. viride* the zone of inhibition is higher in 90 µg/ml concentration (8.56±0.24 mm) against its control (7.76±0.18 mm/ 10µg/ml) (Plate. 2F) followed by 60 µg/ml concentration (4.53±0.21 mm).

3.2 Antioxidant activity

3.2.1DPPH activity

The antioxidant activities of *Solanum melongena* ethanolic and methanolic NaCl salt derived callus extracts were assessed by using DPPH and ABTS activity. The DPPH activity of different concentration of solvent extracts (1 mg/ml to 5 mg/ml) along with standard ascorbic acid is presented in the table 2. Among the five different concentration (50 µg/ml to 250 µg/ml) of extracts tested, the higher percentage of inhibition (52.25 ± 0.41) was observed in 250 µg/ml of methanolic extract followed by ethanolic extract (50.30 ± 0.28), against the standard ascorbic acid (61.53 ± 0.11) (Fig.1). The minimum DPPH activity 6.34 ± 0.09 and 8.12 ± 0.40 was noticed in 50 µg/ml concentration of ethanolic and methanolic extract respectively. The dose titration curves allowed determination of IC_{50} for the ethanolic and methanolic callus extracts towards DPPH scavenging activity. The extracts demonstrated dose dependent DPPH scavenging activity effects with IC_{50} values 248.50 µg/ml, 239.23 µg/ml and 203.15 µg/ml in ethanolic, methanolic extracts and standard ascorbic acid respectively (Fig. 1). The result showed that the both ethanolic and methanolic extracts possess almost similar potent scavenging activity of the stable free radical DPPH.

3.2.2 $ABTS^+$ Scavenging Assay

$ABTS^+$ activity of ethanolic and methanolic extracts of *S. melongena* NaCl salt stressed callus were assayed by using five different concentrations (50 µg/ml to 250 µg/ml). The result of the percentage of inhibition solvent extracts is presented in table 3 and figure 4. The absorbance was increased with the increasing concentrations of both methanolic and ethanolic callus extracts. In this study more inhibition (59.25± 0.49 and 54.90 ± 0.54) was observed in the concentration of 250 µg/ml of methanolic and ethanolic extracts respectively. It is followed by 200µg/ml concentration with 51.48 ± 0.69 and 45.32 ± 0.14 inhibition in methanolic and ethanolic extracts respectively, where as in standard ascorbic acid the absorbance was 72.10 ± 0.47 in 250 µg/ml concentration and 59.10 ± 0.20 in 200 µg/ml concentration (Table 3, Fig. 2). The IC_{50} values of ethanolic and methanolic extracts determined by the values presented in fig 2. IC_{50} values of ethanolic, methanolic and standard ascorbic acid is 227.68 µg/ml, 210.97µg/ml and 169.20µg/ml respectively (Fig. 2). The inhibition value of methanolic and ethanolic salt induced callus extract was almost equal.

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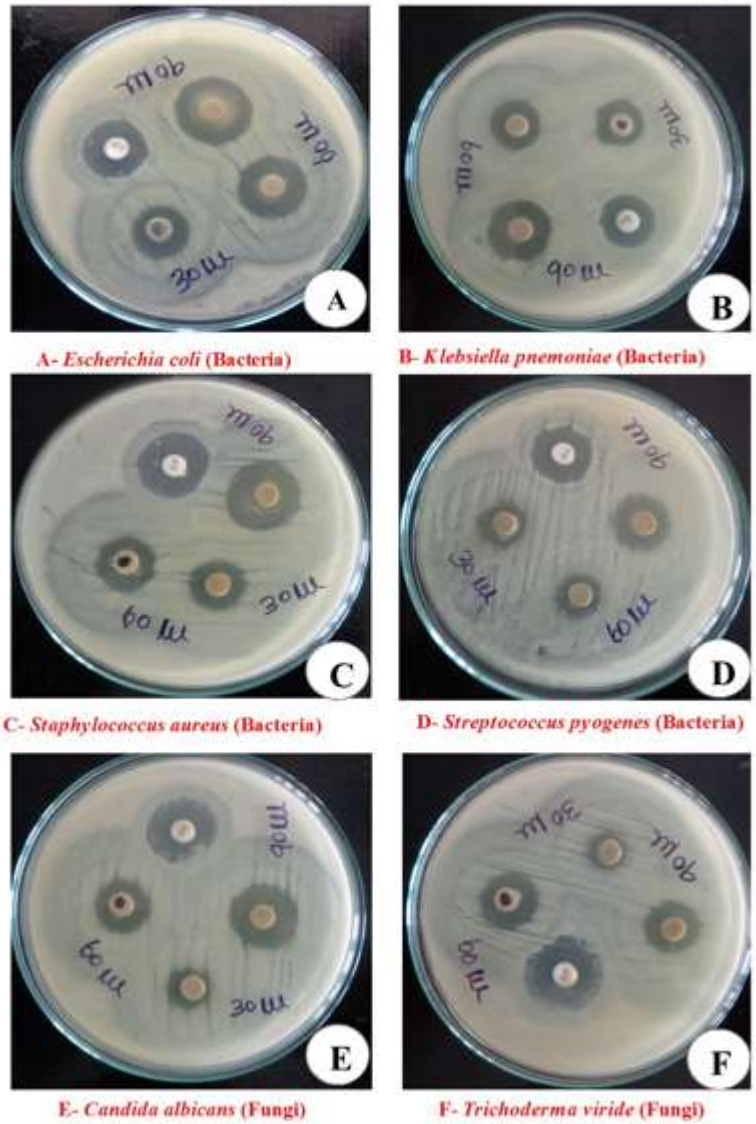
Table.1: Antimicrobial activity of ethanolic and methanolic extracts of Solanum melongena L. callus

S. No	Pathogenic Microbes	Ethanol extract Zone of inhibition (mm)			Standard (Streptocycline)	Methanol extract Zone of inhibition (mm)			Standard (Streptocycline)
		30 µl	60 µl	90 µl		30 µl	60 µl	90 µl	
1.	Escherichia coli	6.53±0.20	9.66±0.17	13.60±0.20	8.50±0.17	6.63±0.20	8.46±0.14	12.50±0.17	8.73±0.08
2.	Klebsiella pneumoniae	5.56±0.24	6.56±0.26	11.83±0.12	7.50±0.23	5.66±0.14	8.53±0.12	12.13±0.26	8.80±0.11
3.	Staphylococcus aureus	6.76±0.14	7.66±0.14	12.70±0.17	10.46±0.14	6.46±0.17	7.50±0.20	12.63±0.14	10.53±0.20
4.	Streptococcus pyogenes	5.46±0.17	6.56±0.20	9.56±0.14	10.53±0.20	6.40±0.15	8.63±0.17	11.23±0.23	10.63±0.14
5.	Candida albicans	3.60±0.26	5.66±0.24	8.63±0.21	8.63±0.12	3.73±0.21	5.56±0.26	8.73±0.21	8.43±0.23
6.	Trichoderma viride	3.63±0.21	5.56±0.08	6.80±0.15	8.70±0.15	3.53±0.23	4.53±0.21	8.56±0.24	7.76±0.18

Plate: 1 Antimicrobial activity of ethanolic extract of *Solanum melongena*



A- *Escherichia coli* (Bacteria) B- *Klebsiella pnemoniae* (Bacteria)

C- *Staphylococcus aureus* (Bacteria) D- *Streptococcus pyogenes* (Bacteria)

E- *Candida albicans* (Fungi) F- *Trichoderma viride* (Fungi)

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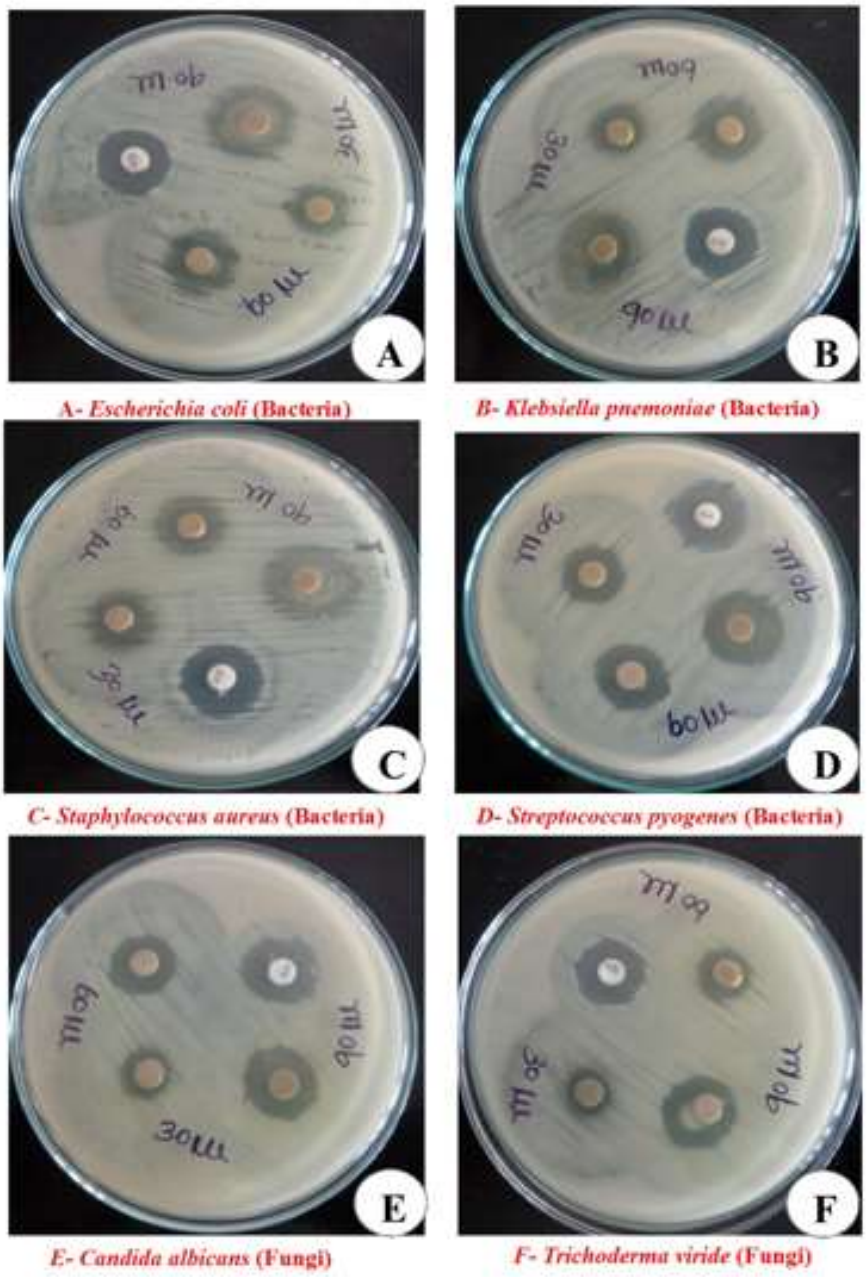
Plate 2- Antimicrobial activity of methanolic extract of *Solanum melongena*



A- Escherichia coli (Bacteria) *B- Klebsiella pneumoniae* (Bacteria)

C- Staphylococcus aureus (Bacteria) *D- Streptococcus pyogenes* (Bacteria)

E- Candida albicans (Fungi) *F- Trichoderma viride* (Fungi)

Table.2: DPPH activity of different concentration of ethanolic and methanolic leaf callus extracts of Solanum melongena L.

S. No.		% inhibition					
		50 (µg/ml)	100 (µg/ml)	150 (µg/ml)	200 (µg/ml)	250 (µg/ml)	IC_{50} Value(µg/ml)
1	Ethanol extract	6.34 ± 0.09	14.64 ± 0.37	22.13 ± 0.38	33.54 ± 0.61	50.30 ± 0.28	248.50
2	Methanol extract	8.12 ± 0.40	16.19 ± 0.52	28.67 ± 0.70	37.29 ± 0.27	52.25 ± 0.41	239.23
3	Ascorbic acid	16.56 ± 0.15	25.76 ± 0.92	37.30± 0.58	49.21 ± 0.20	61.53 ± 0.11	203.15

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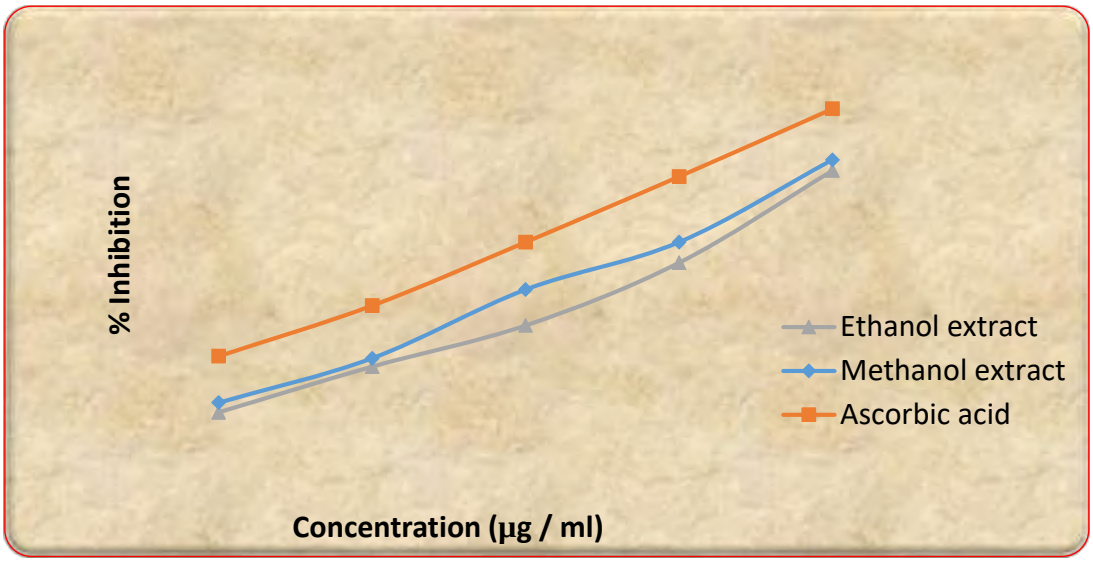

Fig.1:DPPH activity of different concentration of ethanolic and methanolic leaf callus extracts of Solanum melongena L.

IC_{50} value of Ethanol extract : 248.50 µg/ml
IC_{50} value of Methanol extract : 239.23 µg/ml
IC_{50} value of Ascorbic acid (standard) : 203.15 µg/ml

Table.3: ABTS$^+$ activity of different concentration of ethanolic and methanolic leaf callus extracts of Solanum melongena

S. No.		% inhibition					
		50 (µg/ml)	100 (µg/ml)	150 (µg/ml)	200 (µg/ml)	250 (µg/ml)	IC 50 Value(µg/ml)
1	Ethanol extract	11.99 ± 0.09	20.61 ± 0.80	33.85 ± 0.19	45.32 ± 0.14	54.90 ± 0.54	227.68
2	Methanol extract	14.28 ± 0.54	26.10 ± 0. 47	39.45 ± 0.61	51.48 ± 0.69	59.25± 0.49	210.97
3	Ascorbic acid	21.45 ± 0.67	33.39 ± 0.29	47.71 ± 0.62	59.10 ± 0.20	72.10 ± 0.47	169.20

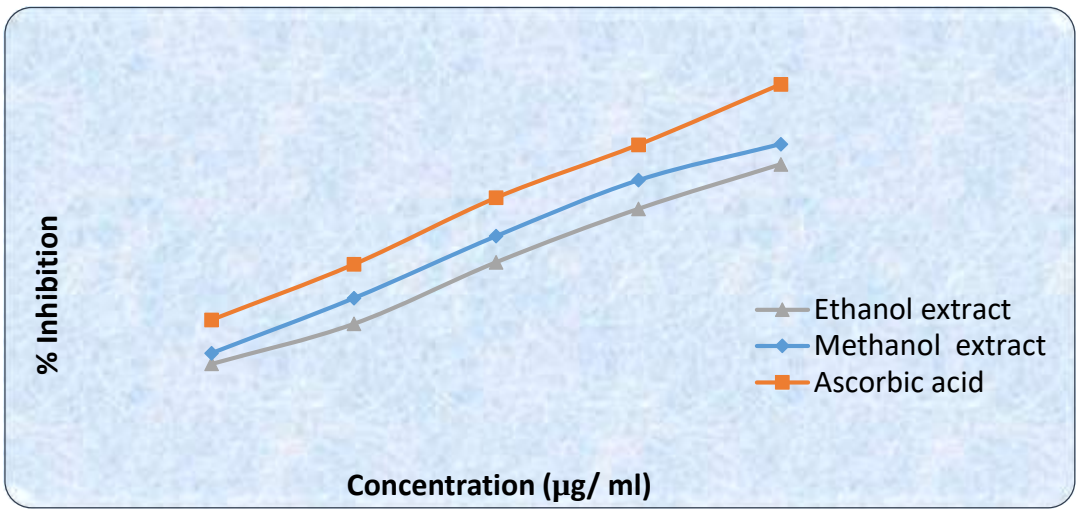

Fig.2: ABTS$^+$ activity of different concentration of ethanolic and methanolic leaf callus extracts of Solanum melongena

IC_{50} value of Ethanol extract : 227.68 µg/ml
IC_{50} value of Methanol extract : 210.97 µg/ml
IC_{50} value of Ascorbic acid (standard) : 169.20 µg/ml

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IV. DISCUSSION

Plants are employed as important source for traditional medications [16]. It is important to study scientifically, plants that have been used in traditional medicines to determine potential sources of novel antimicrobial compounds [17]. Secondary metabolites like alkaloids, glycosides, steroids, flavonoids are potential sources of drugs present in medicinal plants.Moreover the natural antioxidants including carotenoids, flavonoids, cinnamic acids, benzoic acids, folic acid, ascorbic acid, tocopherols and tocotrienols are the secondary metabolites produced by plants for their sustenance. The bioactive substances like Beta-carotene, ascorbic acid and alpha tocopherolare the free radical scavengers with enhanced potential. Natural antioxidants are the vegetables play a significant role in reducing the risk of certain types of cancer, cardiovascular diseases and other chronic diseases [18]. The question of subjecting medicinal herbs to modern scientific test has often been raised. Biosynthesis of secondary metabolites is affected strongly by salt stress resulting in considerable fluctuations in quality and quantity.

Production of secondary metabolites by callus culture have made it possible for the increased yield of a wide variety of pharmaceuticals such as alkaloids, terpenoids, steroids, saponins, phenolics, and flavonoids [19]. Environmental stresses strongly influence plant growth and development. Salinity is one of the most important of these stresses and can limit crop yield [20]. Today, 20% of the World's cultivated land and nearly half of all irrigated lands are affected by salinity [21]. Salt stress has become one of the most damaging environmental hazards to crop productivity all over the world [22].

The cultivation of medical plants using different growth regulators to enhance the production of bioactive compounds is required for commercial and research application. Bioactive compounds were found to be accumulating in culture cells at higher level than those in natural plants though optimization of culture conditions [23]. Free proline and phenol increased exponentially with the increase in NaCl level was reported in *Solanum nigrum*[24].

Antimicrobial effects of ethanolic and methanolic extracts salt callus of *S. melongena* showed good antimicrobial activities against human pathogens. Whereas another reports observed in the leaves of eggplant had antibacterial activity on gram negative only [25]. Whereas, the methanol extract of *S. melongena* seed gave maximum inhibition against *Staphylococcus aureus* and *Escherichia coli.* It is quite obvious that the inhibition level gradually increases in accordance with the level of increase in concentration. Similar observation also found in our results. However, in contrary same methanolic extract of *S. melongena* seed did not have any inhibitory activity against *Pseudomonas aeruginosa*and *Proteus vulgaris* [26].

The steroidal glycoalkaloids are the family of secondary metabolites produced by Solanaceous plants, including potato, tomato and eggplant[27]. Steroidal glycoalkaloids have antimicrobial, insecticidal and fungicidal properties which provide resistance against several insect pests and herbivores[28]. In the present study both the extracts showed good antimicrobial activity. This may be due to the presence of glycoalkaloids present in this family.

In addition to the support our results the mangrove (salt watered growing) plant *Avicennia marina* extracts showed good antimicrobial activity against *E. coli*, *S. aureus*, and *B. subtilis.* The result of present study for antibacterial activity agrees with leaf extract of mangrove plants[29,30,31]. Our reports revealed that the salt stressed *S. melongena* callus extracts shows more antimicrobial activity compared with normal brinjal plant extracts[32].

The DPPH radical were used to study the scavenging activity of some natural compounds. The results of scavenging DPPH radical ability of *S. melongena* at different concentration in comparison with standard ascorbic acid showed in the figure 1. In DPPH scavenging activity assay the IC_{50} value of ethanolic, methanolic and ascorbic acid was 248.50 µg/ml, 239.23 µg/ml and 203.15 µg/ml respectively. The extracts of *S. melongena*showed dose dependent DPPH radical scavenging activity. These findings shows that there is a strong relationship between the secondary metabolites and antioxidant activity of plant materials. Our findings are comparable with earlier reports of *S. melongenain vivo* plant extracts[33] and *S. surathense* leaf extract [34]. The DPPH scavenging activity antioxidants is due to the reaction between antioxidant molecules and radical, which occur by donating the hydrogen during the scavenging of the radical. Our*in vitro* callus extract results were comparable that of *in vivo* plant extract of *S. melongena*and *S.surathense*[33,34].

The decolorization of $ABTS^{+}$cation radical is a way to measure the antioxidant activity of extracts. Positive correlation between phenolic content and antioxidant activity was reported by Awika*et.al.* 2003[35]. Polyphenols are the major plant compounds with antioxidant activity. The activity of phenolic compounds is mainly due to their redox properties[36,37]which can do an main role in free radical absorbing and neutralizing, singlet and triplet oxygen quenching or peroxides decomposing. Result of the present study revealed that ethanolic and methanolic extracts possess superior antioxidant activities. Alcoholic extracts of *S. melongena* showed potent ABTS radical scavenging activity with IC_{50} value of 227.68 and 210.97 µg respectively. Many scientists reported the presence of steroids, terpenoids,

flavonoids, phenolic compounds, tannins in various extracts of *S. melongena*[38,39,40,41]. These reports confirmed that both ethanolic and methanolic extracts showed high level of antioxidant activity in *in vitro* system.

V. CONCLUSION

The presence of various bioactive compounds in the both ethanolic and methanolic extracts of salt stressed callus of *S. melongena* justifies that, the salt stress have been induced to produce strong bioactive compounds. However, isolation of individual phytochemical constituents and subjecting it to the biological activity from salinity tolerance callus will definitely give fruitful results. The results, shows that the salt stressed *S. melongena* callus contains various bioactive compounds. Therefore, it is concluded that *in vitro* clonal propagation with salt stress could alter the biochemical changes which could be a phytopharmaceutically and morphopotencially importance. These reports confirmed that both ethanolic and methanolic extracts showed high level of antimicrobial activity and antioxidant activity in *in vitro* system.

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