Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6 suppl 2, 2014

Research Article

COMPARATIVE ANALYSIS OF PHYTOCHEMICALS, ANTIBIOGRAM OF SELECTED PLANTS IN SOLANACEAE FAMILY AND ITS CHARACTERIZATION STUDIES

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Received: 26 Nov 2013, Revised and Accepted: 10 Jan 2014

ABSTRACT

Objective: *Solanaceae* or nightshades are an economically and medicinally important family of flowering plants .The objective of the work is to identify the phytochemicals, determine the antibiogram in *Solanum esculentum, Solanum trilobatum, Solanum nigrum* and *Solanum tuberosum* and thereby to characterise the bioactive compounds by HPLC and GC-MS analysis

Materials and Methods: Preliminary phytochemicals screening was carried out to identify the presence of various phytochemicals such as tannins, phenols, alkaloids, flavonoids and terpenoids. Antibiogram was estimated by Kirby Bauer method to evaluate the sensitivity of the plant sample under investigation against different pathogens such as *E.coli, Klebsiella pneumoniae* and *Proteus mirabilis.* Further the sample was analysed using techniques such as HPLC and GC-MS to find the active compound responsible for the antimicrobial activity.

Results: Screening revealed the presence of compounds such as tannins, flavonoids, alkaloids, phenols etc. The methanol extract of the plant *Solanum esculentum* showed more antibacterial activity in all bacterial cultures. Instrumentation techniques revealed the presence of compounds such as ferrulic acid, caffeic acid etc.

Conclusion: Based upon the above results it can be concluded that the plants investigated for the current studies exhibits good antimicro bial activity with healthy number of compounds which in turn suggests that *Solanum esculentum* extracts can prevent some of the dreadful pathogenic diseases.

Keywords: *Solanaceae,* Phytochemicals, Antibiogram, HPLC, GC-MS.

INTRODUCTION

Plants are important sources of new antimicrobial agents [1]. Plants are continuously in contact with different microorganisms, including viruses, bacteria and fungi. The relationships established with some of them are beneficial for the plants; thus, some bacteria known as rhizobia, form symbiotic association with leguminous plants by fixing atmospheric nitrogen in root nodules. Other bacteria found close to the plant root (rhizobacteria) are able to control plant diseases caused by soil pathogens [2]. Microorganisms which cause infections are becoming resistant to the drugs that are being commercially used against pathogens causing gastrointestinal disorders [3].

The tomato (*Solanum lycopersicum*, syn. *Lycopersicon lycopersicum* & *Lycopersicon esculentum*) is an herbaceous, usually sprawling plant in the *Solanaceae* or nightshade family that is typically cultivated for the purpose of harvesting its fruit for human consumption.

They contain lycopene, one of the most powerful natural antioxidants. Lycopene has also been shown to improve the skin's ability to protect against harmful UV rays. Tomato is rich in phenolic compounds (flavonoids and phenolic acids), phytoalexins, protease inhibitors, glycoalkaloids and carotenoids, especially lycopene and b-carotene [4].

Solanum nigrum commonly known as black nightshade, grows as a weed, found in the dry parts of India and other parts of the world. It has a long history of medicinal usage and has been used as a traditional folk medicine for treating various ailments such as pain, inflammation, fever and liver disorders. Generally, black nightshade is very rich in nutritive values, which are capable of supplying minerals, vitamins, proteins, and certain hormone precursors.

Solanum trilobatum is extensively used in traditional Indian medicines to cure various human ailments. Solanum trilobatum, a thorny creeper with bluish violet flower, more commonly available in Southern India has been used traditionally in Siddha system of medicines to treat various diseases [5].It was reported that S. Trilobatum possess antioxidant activity hepatoprotective activity [6] and protects UV induced damage and radiation induced toxicity in mice [5].

The potato is a starchy, tuberous crop from the perennial nightshade *Solanum tuberosum*. Potatoes were introduced outside the Andes region four centuries ago, and have become an integral part of much of the world's food supply. It is the world's fourth-largest food crop, following rice, wheat and maize. Long-term storage of potatoes requires specialised care in cold warehouses [7].

Phytochemicals are found in all plant products. It is advised that we consume a wide variety of fruits and vegetables in order to gain maximum benefit from the nutrients and phytochemicals they contain. Preferably, intake of phytochemicals should be from dietary sources rather than from supplements or pills. These can only provide a few of the thousands of phytochemicals available to us and are thus less effective than a serving of fruits and vegetables. [8].

In the year 2008, the anti bacterial activity of saponin isolated from the leaves of *Solanum trilobatom* L. was done against selected bacterial strains, *Staphylococcus aureus*, *Escherichia* coli, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The antibacterial activity was tested by agar disc diffusion and agar well diffusion method. The plant parts tested were extracted with ethanol, acetone and ethyl acetate. Almost all the organic solvent extracts exhibited good inhibitory effect against tested bacterial pathogens. The most susceptible Gram-positive bacterial species was *S. aureus,* while the most susceptible Gram -negative bacteria was *P.aeruginosa.* [9].

In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents [10]. Thus, it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections. Since time immemorial man has used various parts of

plants in the treatment and prevention of various ailments [11]. Because employment of plants to treat diseases renders a good healing with lesser concomitant effects. Hence, this study was aimed to compare the various phytochemicals in *Solanaceae* family and to evaluate the antibiogram of the selected plants including its characterization studies.

MATERIALS AND METHODS

The plant *Solanum esculentum, Solanum trilobatum* and *Solanum nigrum* were collected from in and around villages of Padalam and *Solanum tuberosum* was collected from Ooty. The plant samples were washed with running water twice and dried under shade for 5-15 days. After drying, the plants were ground or crushed to make a dry powder by means of Mortar and pestle. The dried plant powders are soaked in organic solvent methanol. To extract the active compounds they were kept in shaking incubator for 24 hours at 100-150 rpm. After 24 hours the plant samples were filtered through Whatt Man filter paper No.1 and micro filtration unit and the filtered solution was evaporated in rotary vaccum evaporator apparatus. The crude filtrate was dissolved in DMSO and it is centrifuged under optimum speed and the supernatant was collected and stored at 4° C until further use.

Preliminary phytochemical screening was performed on the extracts to detect various phytochemicals such as alkaloids, tannins, flavonoids etc., present in the extract.

Antibiogram tests are used to determine the inhibitory activity of an antibacterial agent existing in the selected plants under study. The rate of diffusion depends on chemical and physical characteristic of the antibiotics. The diameter of the zone of growth inhibition is proportional to the susceptibility of the organism, the inoculum and to the rate of diffusion of the antimicrobial agent. The organisms were grown at 37°C in Mueller Hinton broth.

The Muller Hinton medium was inoculated with ten microliters of the overnight cultures of *E.coli, Klebsiella pneumoniae* and *Proteus mirabilis* further incubated at 37°C with shaking. A sterile swab was dipped into this culture and used to inoculate the surface of a fresh Mueller Hinton agar plates and were allowed to dry for 2 to 5 minutes. The antibiotics disc and the plant sample discs at different concentration were spaced out onto the plates and were incubated at 37°C for 24 hours. The diameter of the clear zone or antibiotic disc and each plant sample discs were determined.

The bioactive compounds were characterised by HPLC and GC-MS technique. For HPLC analysis, the plant extracts were first run through analytical column for the analysis of compounds present. After the analysis of compounds the samples were run through preparative column for the fractionation of compounds. 30 µl of each plant sample is introduced into the stream of preparative column mobile phase. The solvents used in mobile phase are formic acid and methanol. The flow is adjusted to 1 ml/min. Both of the plant samples were run at 340 nm.

These fractions were collected in screw cap tubes. The collected fractions were evaporated by using Heidolph-Flash rotary evaporator and stored at 4°C until use.

GC-MS analysis is a common confirmation test. GC-MS analysis separates all of the components in a sample and provides a representative spectral output. The sample is injected into the injection port of the GC device. The GC instrument vaporizes the sample and then separates and analyzes the various components. Each component ideally produces a specific spectral peak that may be recorded on a paper chart or electronically. The retention time can help to differentiate between some compounds. The size of the peaks is proportional to the quantity of the corresponding substances in the specimen analyzed. The HPLC fractions were analyzed by using GC-MS analysis. The GC-MS column is DB-5. Length, thickness and diameter of the column are 30 m, 0.25 µm and 0.25 mm respectively. Total program run time is 20 min. The temperature program was as follows: injector temperature 250 ºC, initial oven temperature 2 min at 100 ºC, and final oven temperature 20 min at 300 ºC. Transfer line temperature is 250ºC. Helium was used as carrier gas at 68.8 kPa pressure with flow 13.5 ml/min. The GC-MS analysis was done for the HPLC fractions collected at 23.9 min 63.5 min. The GC-MS peaks for fraction 23.9 min were available at retention times 9.6 min, 11.7 min, 12.9 min and 14.91 min. The GC-MS peaks for fraction 63.5 min were available at retention times 6.9 min, 9.3 min and 10.6 min.

RESULTS

The methanol extract of the plants from *solanaceae* family indicates the presence of various phytochemicals such as tannins, flavonoids, alkaloids, phenols, steroids, glycosides and saponins. Table 1.demonstrates the results for phytochemical screening of four different plant extracts of *solanaceae* family.

The antibacterial activity of the *Solanum esculentum, Solanum trilobatum, Solanum nigrum* and *Solanum tuberosum* plant species extracts were assayed in vitro by agar disc diffusion method against three bacterial species like *E.coli, Klebsiella pneumoniae* and *Proteus mirabilis* and tabulated in table 2. The methanol extract of the plant *Solanum esculentum* showed more antibacterial activity in all bacterial cultures when compared to *Solanum trilobatum, Solanum nigrum* and *Solanum tuberosum* plant extracts. The figures (plates) 1, 2, and 3 showed the inhibitory effect of methanol extract of *Solanum esculentum* variety against *E.coli, Klebsiella pneumoniae* and *Proteus mirabilis* respectively.

Table 1: Qualitative analysis of phytochemicals in various selected plants from of *Solanaceae* family

Phytochemicals	*Solanum esculentum*	*Solanum trilobatum*	*Solanum nigrum*	*Solanum tuberosum.*
Alkaloids	+	+	+	+
Steroids	+	-	+	-
Triterpenoids	-	-	-	-
Flavonoids	-	-	+	-
Tannins	+	+	+	+
Phenols	+	-	+	+
Glycosides	+	+	+	+
Saponins	+	+	+	+
Phlobotannins	-	-	-	-
Anthraquinones	-	-	-	-

Table 2: antibacterial activity of methanol extract of *solanum esculentum*, *solanum trilobatum*, *solanum nigrum* and *solanum tuberosum* against various bacterial species

S. No.	Micro organism	Zone of inhibition in mm				
		1000 µg	500 µg	250 µg	100 µg	Streptomycin 10 µg
Sample 1						
Solanum esculentum						
1	*E.coli*	30	20	18	12	25
2	*Klebsiella pneumonia*	20	15	12	10	20
3	*Proteus mirabilis*	10	8	7	8	15
Sample 2						
Solanum trilobatum						
4	*E.coli*	11	10	9	8	25
5	*Klebsiella pneumonia*	10	8	9	8	20
6	*Proteus mirabilis*	9	7	6	5	12
Sample 3						
Solanum nigrum						
7	*E.coli*	15	10	8	8	25
8	*Klebsiella pneumonia*	10	9	8	9	20
9	*Proteus mirabilis*	8	6	7	8	12
Sample 4						
Solanum tuberosum						
10	*E.coli*	12	9	10	9	25
11	*Klebsiella pneumonia*	9	8	8	7	20
12	*Proteus mirabilis*	8	7	8	8	12

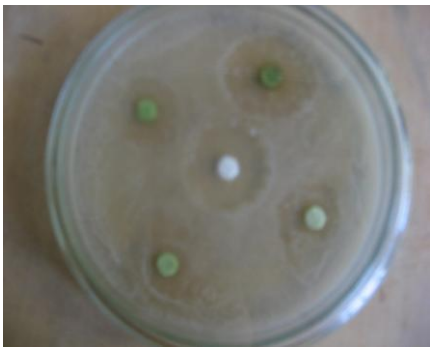

Fig. 1: The inhibitory effect of methanol extract of *Solanum esculentum* variety against *E.coli*

Fig. 2: The inhibitory effect of methanol extract of *Solanum esculentum* variety against *Klebsiella pneumonia*

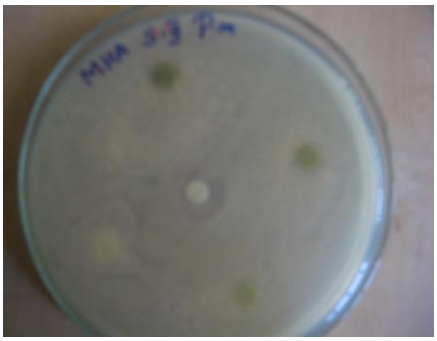

Fig. 3: The inhibitory effect of methanol extract of *Solanum esculentum* variety against *Proteus mirabilis*

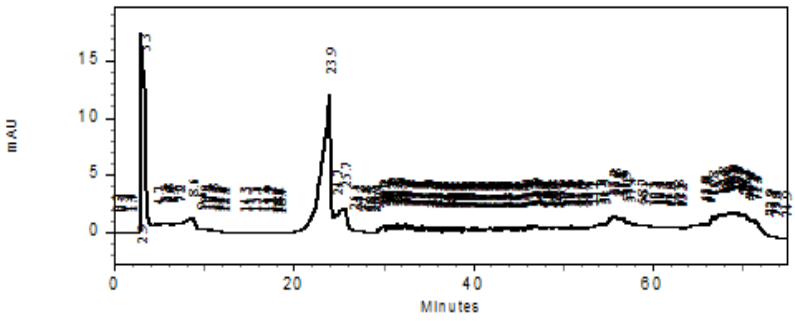

Fig. 4: The fractionation of methanol extract of *Solanum esculentum*

The compounds were analyzed and isolated by HPLC technique. The figure 4 shows the fractionation of methanol extract of *Solanum esculentum*. The *Solanum esculentum* plant sample gave peaks at different time intervals like 3.1 min, 23.9 min and 63.5 minutes.

The *Solanum trilobatum* plant sample gave peaks at 3.7 min, 24.7 min and 60.5 minutes.The *Solanum nigrum* plant sample gave peaks at 3.5 min and 28.5 min. The *Solanum tuberosum* plant sample gave peaks at 2.7 min, 35.7 min.

From the figures, it is clear that the chromatogram graph of *Solanum esculentum* shows three peaks which indicate the presence of more bioactive compounds than that of *Solanum trilobatum, Solanum nigrum* and *Solanum tuberosum*. Further identification of compounds from *Solanum esculentum* was done by UV-Vis spectrophotometer and GC-MS analysis. The UV-vis spectrum graph for *Solanum esculentum* fraction collected at different retention times were given in the figures 5 and 6.

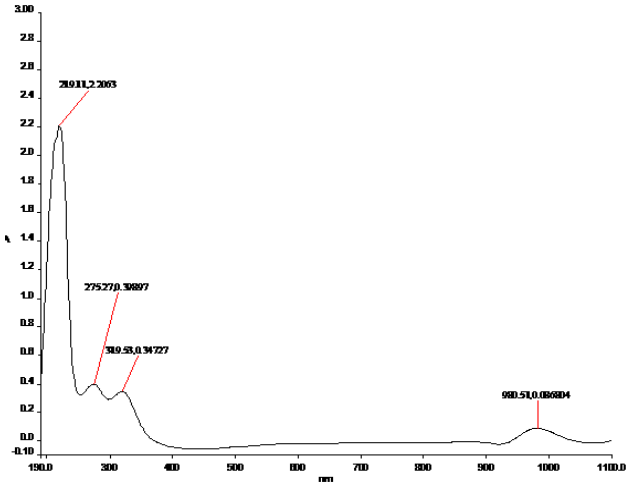

Fig. 5: The UV spectrum graph for *Solanum esculentum* fraction collected at 3.1 min

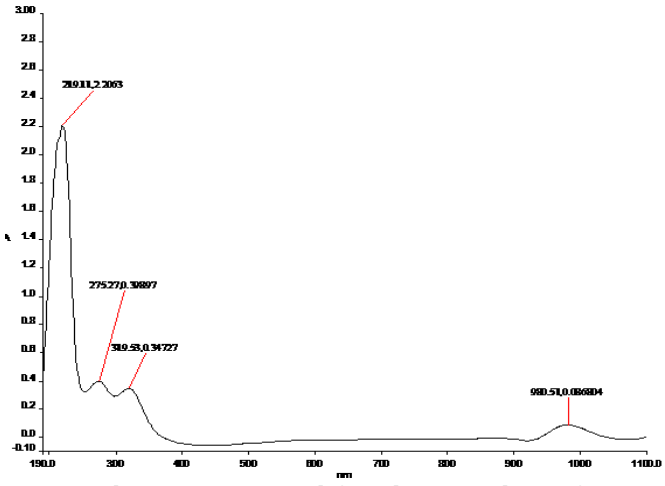

Fig. 6: The UV spectrum graph for *Solanum esculentum* fraction collected at 23.9 min

The GC-MS analysis was done for the *Solanum esculentum* fractions collected at 3.1 min and 23.9 min

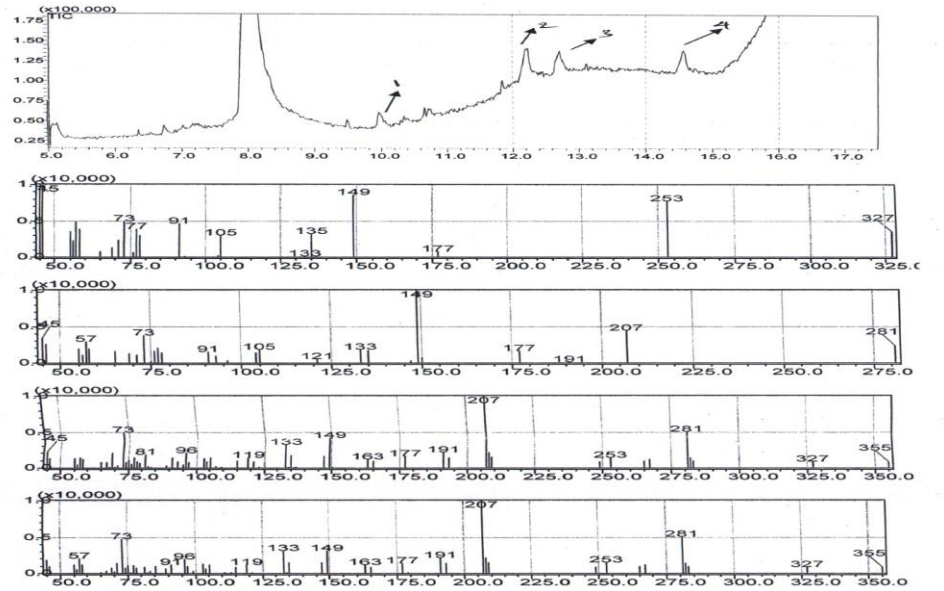

Fig. 7: GC-MS graph for the *Solanum esculentum* fraction collected at 3.1 min

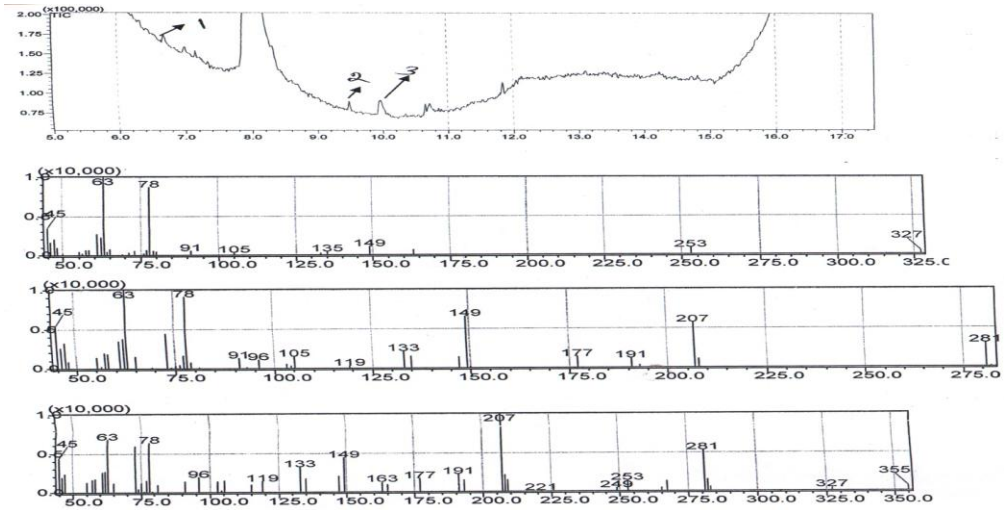

Fig. 8: GC-MS graph for the *Solanum esculentum* fraction collected at 23.9 min.

DISCUSSION

The Solanum family plant contains bioactive compounds like alkaloids, flavonoids, steroids and saponins etc.[12]. They are having antimicrobial activity against some bacterial species like *E.coli, Klebsiella pneumoniae,* and *Proteus mirabilis* [13]. The antibacterial activity was assayed by the standard Kirby-Bauer method (Antibiotic Susceptibility Test).The assay was carried out with a standard antibiotic Streptomycin as positive control. The drug of our test plants are diluted to four different concentrations for the assay. The methanol extract of *Solanum esculentum* shows reasonable antibacterial activity. The compounds were identified by using GC-MS analysis and HPLC analysis. From the GC-MS results, the compounds found in *Solanum esculentum* plant fractions were identified as Caffeic acid, Ferulic acid, *p*-Coumaric acid-*O*-*â*-d-glucoside and Kaempferol.[14]

CONCLUSION

The Preliminary phytochemical investigation reveals the presence of various important phytochemicals such as alkaloids, tannins and saponins The antibacterial activity of selected Solanaceae family plants *Solanum esculentum*, *Solanum trilobatum*, *Solanum nigrum* and *Solanum tuberosum* were determined against bacterial strains. The methanol extract of the plant *Solanum esculentum* highly inhibits all the bacterial strains investigated.

The bioactive compounds were isolated from the plant *Solanum esculentum*. The methanol extract of *Solanum esculentum* plant contains more fractions. Further works carried out on *Solanum esculentum* plant fractions and the bioactive compounds were identified using GC-MS. The identified bioactive compounds are Caffeic acid, Ferulic acid, *p*-Coumaric acid-*O*-*â*-d-glucoside and Kaempferol. They are responsible for the antibacterial activity of the plant *Solanum esculentum*. The bioactive compounds were isolated from the edible part (leaves) of the plants. They are used in laboratories for the research purpose and also they are used in the medicinal field.

REFERENCES

1. Jigna Parekh, Sumitra V.Chanda. In vitro Antimicrobial Activity and Phytochemical Analysis of some Indian Medicinal Plants. *Turk. J. Bio*. (2007); 31: 53-58.
2. Bais, H.P.,Weir, T.L., Perry, L.G., Gilroy, S. Vivanco, J.M. The role of root exudates in rhizosphere interactions with plants and other organisms. *Annu. Rev. Plant Biol.* (2006); 57: 233-266.
3. Srividhya M, Ramanathan K, Krishnanand N. Efficacy of citrus fruit peel extracts against pathogens causing gastro intestinal disorders. *Int.J. Pharm Pharm Sci.* (2013); Vol 5: 160-163.
4. Rajkumar S, Jebanesan A. Ovicidal activity of *Solanum trilobatum* Linn (Solanaceae); leaf extract against *culex quinquefasciatus* Say and *Culex triaeniorhynchus* Gile (Diptera: Culicidae. *Intl. J. Trop. Sci.* (2004); 24: 340-342.
5. Amir M, Kumar S. Possible Industrial application of genus *Solanum* in twentyfirst century. *J. Sci. Ind. Res.* (2004); 63: 116-124.
6. Shahjahan M, Sabitha KE, Mallika Devi R, Shyamala CS. Effect of medicinal plants on tumourogenesis. *Ind. J. Med. Res.* (2004); 123: 23-27.
7. Tokusoglu O., Unal M.K., Yildirim Z. HPLC-UV and GC-MS characterization of the flavonol aglycones Quercetin, Kaempferol and Myricetin in Tomato pastes and other tomato-based products. *Acta Chromatograph.* (2003); 13: 196-207.
8. Harbone JB. Phytochemical methods. *Chapman and Hill* (1973).
9. Swapna Latha P. and K. Kannabiran, Antimicrobial activity and phytochemicals of *Solanum trilobatum* Linn.*Afr. J. Biotech.* (2006); 5: 2402-2404.
10. Vandana Mathur, Sharad Vats, Megha Jain, Jaya Bhojak and Raka Kamal. Antimicrobial Activity of Bioactive Metabolites Isolated from Selected Medicinal Plants. *Asian J. Expt. Sci.* (2007); 21: 1-6.
11. Tanaka H, Sato M, Fujiwara S. Antibacterial activity of isoflavonoids isolated from *Erythrina variegata* against methicillin-resistant *Staphylococcus aureus*. *Lett. Appl. Microbiol.* (2002); 35: 494-498.
12. Nicoletti I, Bello C, De Rossi A, Corradini D. Identification and quantification of phenolic compounds in grapes by HPLC-PDA-ESI-MS on a semimicro separation scale. *J. Agri. Food Chem*. (2008); 56: 8801-8.
13. Toor RK, Savage GP. Antioxidante activity in different fractions of tomato. *Food Res. Intl*. (2005); 38: 487-494.
14. Tokusoglu O., Unal M.K., Yildirim Z. HPLC-UV and GC-MS characterization of the flavonol aglycones Quercetin,
15. Kaempferol and Myricetin in Tomato pastes and other tomato-based products. *Acta Chromatograph.* (2003); 13: 196-207