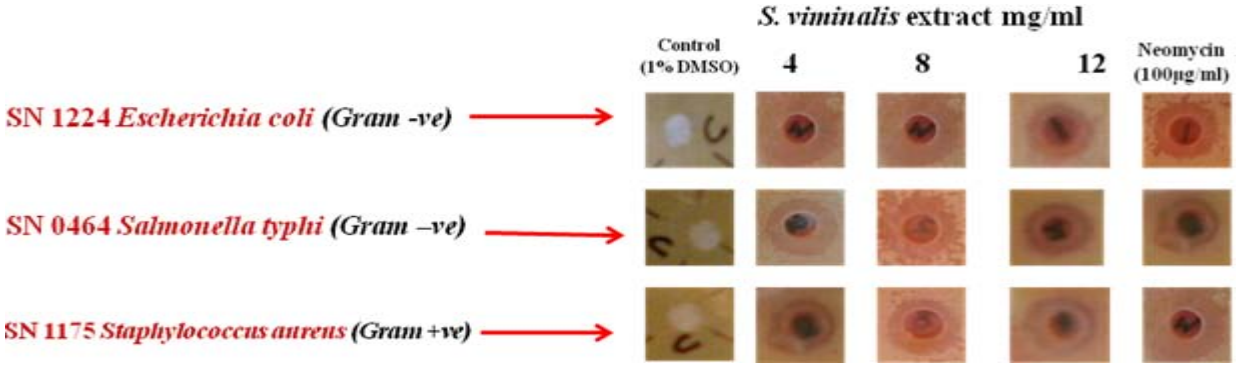


Figure 1: Photographs obtained in filter disc assay of the test extract against *Escherichia coli* SN 1224 *(Gram -ve)*, *Salmonella typhi* SN 0464 *(Gram –ve)* and *Staphylococcus aureus SN 1175 (Gram +ve).*

Table 4: Anti-bacterial activity screening data for different test extract concentrations and Neomycin

Zone of Inhibition (mm)			
Test extract	*SN 1224 Escherichia coli (Gram -ve)*	*SN 0464 Salmonella typhi (Gram –ve)*	*SN 1175 Staphylococcus aureus (Gram +ve)*
4mg/ml	3.31±0.11	5.65±0.18	4.11±0.50
8mg/ml	8.65±0.49	9.65±0.40	10.23±0.35
12mg/ml	10.15±0.81	**12.80±0.75**	11.40±0.72
Neomycin [a] (100µg/ml)	14.16±0.75	15.59±0.57	15.59±0.49
Control [b] (1% DMSO)	-	-	-

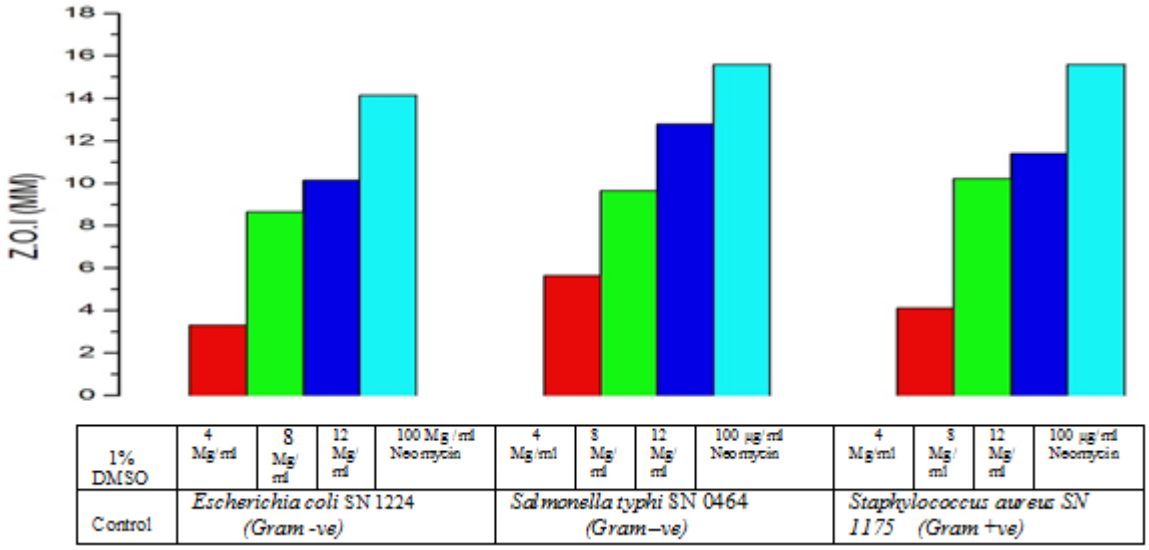
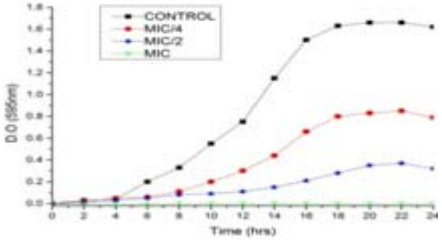


Figure 2: Bar diagram showing comparison between anti-bacterial activities of Petroleum ether leaf extracts of *S. viminalis* at different concentrations and standard anti-bacterial drug against **(a)** *Escherichia coli* SN 1224 *(Gram -ve)* **(b)** *Salmonella typhi* SN 0464 *(Gram –ve)* **(c)** *Staphylococcus aureus SN 1175 (Gram +ve)*

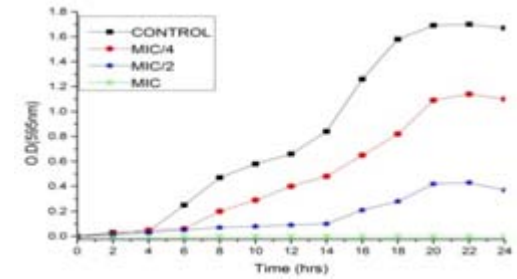
4.2 Growth Curve Studies

(Turbidness Measurement):

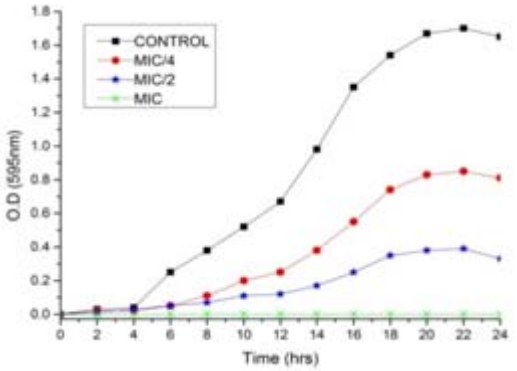
Growth curve of the *bacterial* species was investigated at different concentrations of Petroleum ether leaf extracts of *S. viminalis.* Figure 3a, 3b & 3c with dissimilar concentrations of Petroleum ether leaf extracts of *S. viminalis* showed different effect on growth pattern of *Escherichia coli* SN 1224 *(Gram -ve)*, *Salmonella typhi* SN 0464 *(Gram –ve)* and *Staphylococcus aureus SN 1175 (Gram +ve)*. With the lag phase of 4 hrs control cells showed a normal growth & active exponential phase in 8-10 hrs before reaching last phase. The culture reached the stationary growth phase after 16 hrs. In case of control cells as indicated by optical density it showed normal curve. Increase in the concentration of the extract showed decrease in growth with concealed and deferred exponential phase in comparison to control. Initially this showed lag phase, then exponential phase and at last a stationary phase. No growth was seen which is shown by smooth line at minimum inhibitory concentration values.



(a) *Escherichia coli* SN 1224 *(Gram -ve)*



(b) *Salmonella typhi* SN 0464 *(Gram –ve)*



(c) *Staphylococcus aureus SN 1175 (Gram +ve)*

Figure 3: Effect of different concentrations of Petroleum ether leaf extracts of *S. viminalis* on growth of different bacterial species. Growth curve pattern against absorbance at 595nm (hrs) shows complete inhibition of growth at MIC values **(a)** Against *Escherichia coli* SN 1224 *(Gram -ve)* **(b)** Against *Salmonella typhi* SN 0464 *(Gram –ve)* **(c)** Against *Staphylococcus aureus SN 1175 (Gram +ve)*

5. Discussion

The potential for obtaining antimicrobial agents from medicinal plants seems satisfying, as it will guide to the enhancement of natural medicine to be used against different pathogens. Our findings provide an idea for intensifying the efficacy of plant active principals as anti-bacterial agents. According to the results Petroleum ether leaf extracts of *S. viminalis* exhibited antibacterial activity, shown by filter disc assay & growth curve study against *Escherichia coli* SN 1224 *(Gram –ve)*, *Salmonella typhi* SN 0464 *(Gram –ve)*, *Staphylococcus aureus SN 1175 (Gram +ve*. As a function of various concentrations growth kinetic studies also pursue the similar trend while as tested cells of MIC/4 shows dejected expansion curves with clearly distinguished growth phases. MIC/2 treated cells revealed concealed and late exponential growth phase. Finally at MIC value S shaped growth curve reduced to smooth (flat) line viewing nearly complete death of cell growth (Fig. 3). Filter disc assay solid media revealed efficient inhibition of growth of different *bacterial* isolates with the test extract and was found to enhance in absorption dependent approach Figure 1.

6. Conclusion

Petroleum ether leaf extracts of *S. viminalis* has revealed potent anti-bacterial effect in both solid & liquid medium. This research work is a supplementary attempt for development of new therapeutic agents which is anti-bacterial, less poisonous & helps in prevention of drug resistance. Additional examination and testing needs to be done which is very necessary, that may help to make possible applications of this extract in future as anti-bacterial agent.

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References

[1] Nostro A, Germanò MP, D'angelo V, Marino A, Cannatelli MA. Extraction methods and bio autography for evaluation of medicinal plant antimicrobial activity. Lett Appl Microbiol. 30:379-384, 2000.
[2] Arokiyaraj S, Radha R, Martin S, Perinbam K. Phytochemical analysis and antidiabetic activity of Cadaba fruticosa R.Br. Indian J Sci Technol. 1:1e4, 2008.
[3] Gangadevi V, Yogeswari S, Kamalraj S, Rani G, Muthumary J. The antibacterial activity of Acalypha indica L. Indian J Sci Technol. 1:1-5, 2008.
[4] Patil Bhagaman, Rajput A. J Pharm Res. 5:1228-123, 2012.
[5] R. D. Meikle, Willows and Poplars of Great Britain and Ireland, BSBI Handbook, Vol. 4, 1984
[6] J.G. Mahdi, A.J. Mahdi and I.D. Bowen. The historical analysis of aspirin discovery, its relation to the willow tree and anti-proliferative and anticancer potential. Cell Prolif. 39, 147-155, 2006.
[7] B. Schmid, B. Tschirdewahn, I. Katter, I. Gunaydin, R. Ludtke, H. K. Selbmann, W. Schaffne and L Heide. Analgesic Effects of willow bark extract in osteoarthritis, Results of a clinical double-blind Trial. Focus Alternat. Complement. Ther. 3, 1-86 1998.
[8] V. P. Makhlayuk, Medicinal Plants in Folk Medicine [in Russian], Privolzh. Kn. Izd., Saratov, 1967.
[9] I. E. Akopov, Hemostatic Plants, Meditsina, Tashkent in Russian, 1981.
[10] J. Lauriault, Identification Guide to the Trees of Canada. Fitzhenry and Whiteside, Ontario, 1989.
[11] M. A. Weiner, Earth Medicine, Earth Food: Plant Remedies, Drugs, and Natural Foods of the North American Indians, 1991.
[12] J. A Vinson, y. Hao, X, Su, L. Zubik Phenol antioxidant quantity and quality in foods: Vegetables. J agric food chem, 46, 3630-3634, 1998.
[13] S. P. Pohjamo, J. E. Hemming, S. M. Willfor, M. 0H. Reunanen and B. R. Holmbom, Phenolic extractives in *Salix caprea* wood and knots. Phytochem. 63, 165-169, 2003.
[14] M. S. Alam, G. Kaur, Z. Jabbar, K. Javed and M. Athar, Evaluation of antioxidant activity of *Salix caprea* flowers. Phytother. Res. 20, 479-483, 2006.
[15] Slepecky RA, Leadbetter ER: Ecology and relationships of endospore-forming bacteria: Changing perspectives. In Piggot P, Moran Jr, CP and Youngman P (eds).

Regulation of Bacterial Differentiation. Am Soc Microbiol Press, 1994.
[16] Jurtshuk P Jr, Mueller TJ, Acord WC. Bacterial terminal oxidases. Crit Rev Microbiol. 3:359, 1975.
[17] Feng, Peter, *et al*., "Enumeration of *Escherichia coli* and the Coliform Bacteria," in Bacteriological Analytical Manual 8th Ed. 2002.
[18] Eisenstein, Barry and Zaleznik, Dori, "Enterobacteriaceae," in Mandell, Douglas, & Bennett's Principles and Practice of Infectious Diseases, Fifth Edition, Chap. 206, pp. 2294-2310 (2000).
[19] James M. Jay, Modern Food Microbiology, 6th ed. 2000.
[20] Black PH, Kunz LJ, Swartz MN. Salmonellosis—a review of some unusual aspects. N Engl J Med. 262:811, 864, 921, 1960.
[21] Chopra AK, Peterson JW, Chary P. et al. molecular characterization of an enterotoxin from *Salmonella typhimurium*. Microb Pathogen. 16:85, 1994.
[22] Eisenhauer N, Klier M, Partsch S, et al. No interactive effects of pesticides and plant diversity on soil microbial biomass and respiration. Appl Soil Ecol. 42:31-36. 2009.
[23] Kell DB, Brown M, Davey HM, Dunn WB, Spasic I, Oliver SG. Metabolic foot printing and system biology: the medium is the message. Nat Rev Micribiol. 3:557-565, 2005.
[24] Brindha P, Sasikala B, Purushothaman KK. Pharmacological studies on Merugan kizhangu. Bull Medico Ethnobot Res. 3:84-96, 1981.
[25] Simione, F.P. Key issues relating to the genetic stability and preservation of cells and cell banks. J Parenter Sci Technol 46:226-32, 1992.
[26] Z. S. Sadiq M., K. Fehmeeda. Chloroform leaf extract of *Salix alba* is a rich source of antimicrobial agents of enormous clinical importance EAR, vol. 2, Issue 9.dec; pp: 10098-10115, 2014.
[27] Z. S. Sadiq M, A. Nida, S Shreaz, R. Bhatia and K. Fehmeeda. Phytochemical analysis and Growth Inhibiting effects of *Salix viminalis* L. Leaves against different Candida isolates, ASL, vol. 20. pp. 1463-1467(5), 2014.

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