

Physicochemical and Phytochemical Screening and Antimicrobial Activity of Clerodendrum infortunatum Linn. Species Used for Wound Healing

A. M. Shrirame¹, S. R. Hiwale², L. L. Sawarkar³, S. B. Wadaskar⁴

Department of Botany, KZS Science College, Bramhani, Kalmeshwar, Nagpur, Maharashtra, India¹⁻³

Department of Botany, Bhagwantrao Arts and Science College, Etapalli, District Gadchiroli, Maharashtra, India⁴

srh.bot.kzs@gmail.com² and sawarkarlalita9@gmail.com³

Abstract: The plant body of *Clerodendrum infortunatum* Linn. belong to the family verbanaceae is shrubby and slightly woody 1-2m height, often expressive, branched with blunt quadrangular stem. Flowers are light bluish purple frequently white with terminal panicles in inflorescence. The Leaves are ovate, cordate, acuminate, entire or seriate. The phytochemical study of leaves material showed that, the various chemical compound have been detected such as viz. amino acids phenols, phenols, flavonoids, diterpenoids, triterpenoids, coumarins, steroids and also was found greater antimicrobial activity against various bacteria. On the basis of this research output it concluded that this plant would be remedy for wound healing treatment.

Keywords: *Clerodendrum infortunatum* Linn. leaves materials, phytochemical analysis, bioactive compounds, Thin layer chromatography, bacterial species, antibacterial activity.

I. INTRODUCTION

Current traditional medicine is widely practiced, especially in developing countries. As well as the fact that traditional health care is highly required after in terms of certain cultural elements in the lives of these individuals within these societies (Taylor, J. L. S. et al., 2001). The tribal community depends for their medical aid on local and traditional healer existing near habitat. *Clerodendrum infortunatum* species were found in their local habitat. It is a terrestrial shrub occurring throughout the plains of India. The roots and leaves of *Clerodendrum serratum* L., were with high wound healing potency was studied on Albino rats. The leaves extracts of *Clerodendrum infortunatum* L., was evaluated for its wound-healing potency in experimental rats shows good efficiency (Vidyai, S.M. et al., 2005; Gouthamchandra, K. et al., 2009 and Sannigrahi, S. et al., 2009). The number of the patients interviewed in villages for using *C. infortunatum* L., as a herbal medicines to cure wounds and burns (Korpenwar, A. N., 2012). Many species of the genus *Clerodendrum* showed potent anti-inflammatory activity. *C. phlomidis* was reported by Surendrakumar, P. (1988). Similarly *C. petasites* was reported to show moderate anti-inflammatory activity in the acute phase of inflammation in rats (Panthong, D., et al., 2003).

The ethnobotanical study revealed on traditional uses of the plants by various indigenous communities and identified phytochemicals have been observed on biological activities of the plant. On the basis of this study proved that the plant *C. infortunatum* is suitable for the development of therapeutic agents against various ailments or disorders by Prashith Kekuda, T.R. (2019). As per the scientific study (Kar, P. 2014) of *Clerodendrum* spp. perform multiple pharmacological and therapeutic activities such as anti-microbial, anti-inflammatory, anti-malarial, anti-diabetic, anti-cancer, analgesic. Katewa, S.S. et al. (2004), stated that the patients suffering from wounds are cured by local application of leaf paste or juice of *Clerodendrum infortunatum* L., thrice a day for healthcare delivery. These natural



agents induce healing and regeneration of the tissue by multiple mechanisms, however, there is need for scientific validation, standardization and safety evaluation of plants of traditional medicine before they could be recommended for healing of wounds (Bennet, R. G., 1988; Omale, J. and Victoria, I. A., 2010; Kaleab, A. and Adem, Y., 2013 and Santhosh, A. M., et al., 2015). As per study (Tambe, and Bhambar, 2014), substances such as proteins and minerals, secondary metabolites are having huge capacity to fight against microbes. The report on Leaves, roots and flowers of *C. infortunatum* are used as medicine in the form of paste, juice and ash. Roots are used to treat bronchitis and asthma in Assam (Kalita, N. and Kalita, M.C. 2014). Dhal, P. et al. (2018), investigated that ethanolic leaf extract of *C. philippinum* was rich in phytochemical constituents (alkaloids, flavonoids, glycosides and terpenoids) and also showed presence of antimicrobial properties against some pathogenic strains of bacteria. The phytochemicals study on *Clerodendrum* genus, the aerial parts, roots and leaves were the most common bioactive principles and most of these compounds were reported from *C. serratum*, *C. inerme*, *C. bungei*, *Clerodendrum incisum*, *C. infortunatum*, and *C. trichotomum* such as Diterpenoids, flavonoids, phenylethanoid glycosides, and steroids are abundant Wang, J.H. et al. (2018). Arokiyaraj, S. et al. (2012), reported that the methanolic extract of leaves of *C. siphonanthus* exhibited significant antibacterial effect against *Klebsiella pneumoniae*, *Proteus mirabilis*, *Salmonella typhi*, *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilis*.

The present investigation is aimed to investigate and characterize the leaves material of *Clerodendrum infortunatum* L., for qualitative analysis.

II. MATERIALS AND METHODS

The fresh leaves of plant material was collected and prepared for experimental analysis (**Fig. no.1**).

Organoleptic properties : These properties could be determined through sensory organ such properties as color, taste, and smell. It shows the physical description of the plant powders. These properties were studied by Kokate, C. K. et al., 2002; Patil, S. G., 2013 and Khandelwal, K.R. and Sethi, V., 2012.

Physicochemical properties : These properties could be determined the identity, purity and quality of plant powders. These properties were studied by according to WHO guidelines (Kokate, C. K. et al., 2002 and Sharma, M. et al., 2013). These properties such as ash content (total ash and acid insoluble ash), extractive value (water soluble and alcohol soluble extractive), moisture content and foreign matter were quantified.

Qualitative Studies on Chemical Constituents of Selected Plant Species

Secondary phytochemical screening was done according to the standard procedure adopted by the Stahl, E. (1969); Harborne, J. B. (1973 and 1998); Wagner, H. and Bladt, S. (1996); Thimmaiah, S. R. (1999); Egger, K., (1969); Bray, W.J. et al. (1977); Daniel (1991); Stevens, (1968).

Qualitative analysis of various phytochemicals such as amino acids (free and bound), phenols, flavonoids, diterpenoids, triterpenoids, coumarins, anthraquinones and anthracene derivatives, steroids were carried out from plant material by using **thin layer chromatographic technique**.

Test microorganisms : Human pathogenic organism (bacteria) isolated from clinical samples were used in this study. The organism used were *Acinetobacter* sp. obtained from pathology lab, *Klebsiella* sp. obtained from pathology lab, *S. aureus* (ATCC25923), *E. coli* (ATCC25922), *Enterobacter* sp. (MDR), *Bacillus cereus* (ATCC 10876), *Serratia* sp. (MDR) as well as streptomycin and gentamycin as a control were used.

Agar- Well Diffusion Method: The antimicrobials agent present in the plant extract are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimetres (Mbata et al., 2006 and Norrel, and Messley, 1997).



III. RESULT AND DISCUSSION

Organoleptic properties: Organoleptic characters such as colour, odour and taste of plant powders revealed the preliminary identification of the sources of plant materials with the help of sensory organs. The plant materials of *Clerodendrum infortunatum* L., was green in color, odour with bitter in taste (table no.1)

Physicochemical properties: These properties determined the identity, purity and quality of plant powders. It includes ash content (total ash and acid insoluble ash), extractive value (water soluble and alcohol soluble), and moisture content and foreign matter (table no.2)

a) Ash value: The ash content in *Clerodendrum infortunatum* L., was 11.99%. The previous result about total ash were shows in *Clerodendrum serratum* L. (12.54 %), was reported by Shukla, N. et al.(2015).

The acid insoluble ash value in *Clerodendrum infortunatum* L., 3.99%. The earlier worked shows as, in *Clerodendrum serratum* L. the acid insoluble ash (3.62 %), was reported by by Shukla, N. et al. (2015), the values very closer to each other between two species.

b) Extractive value

The water soluble and alcohol soluble extractive value was found in *Clerodendrum infortunatum* L. as (22.30% & 24.37%), where as in *Clerodendrum serratum* L., water soluble extractives (22.45 %), and alcohol soluble extractives (6.49 %) were reported by Shukla, N. et al. (2015).

c) Moisture content / Loss on drying: The results shows the amount of moisture content in *Clerodendrum infortunatum* L. as (8.05%).

d) Foreign materials: The foreign materials content of *Clerodendrum infortunatum* L. as (1.89%). According to API part-I (Volume-III-2008) the foreign matters should not be more than 2%.

Qualitative Analysis by Thin layer chromatography of selected plant species: Eight major chemical compounds were qualitatively analyzed by thin layer chromatography of selected plant species i.e. amino acids, phenols, flavonoids, anthracene derivatives, coumarins, diterpenoids, triterpenoids and steroids and the results obtained they were summarized in table no 3.(**Fig. no. 2 & 3**)

Twelve different Rf values of amino acids (free and bound) were detected from the plant materials. All obtained Rf values were compared with the 22 standards amino acids. The depicted Rf values as Butyric Acid, Arginine, Cystein, iso-leucine, lysine, methionine, Ornithine, Phenyl alanine, Proline, Threonine, Tyrosine, Valine . The detection of phenol with four Rf value (0.05, 0.23, 0., 0.80 and 0.97) were detected with blue bands. The flavanoids with two Rf values were found (0.61 and 0.88) with Yellow bands. The anthraquinones and anthracene having five different Rf values were spotted (0.10, 0.14, 0.54, 0.74, and 0.88) with Yellow and red bands. The coumarins having two different Rf values were observed (0.73 and 0.78). The diterpenoids having four different Rf values were detected in (0.11, 0.50, 0.75 and 0.94). The triterpenoids having two different Rf values was found (0.13 and 0.98) and steroids having two Rf values were detected (0.25 and 0.90).

Flavonoids, Triperpenoids and steroids was observed from *Clerodendrum infortunatum* L., through TLC reported by Acharya, N. S. and Patel, J. J. (2016).

About anti-bacterial activity shows, the *Clerodendrum infortunatum* L. with chloroform extract against *Escherichia coli*, *Acinetobacter* sp., *Klebsiella* sp., *Staphylococcus aureus*, *Serratia* sp., *Enterobacter* sp. have an high inhibitory effect against all the microorganisms except against *Bacillus cereus* no such activity was found., while in methanol extract showed high inhibition activity except against *Serratia* and , *Enterobacter* sp. no such inhibitin zone was found.

In case of methanolic extract of *Clerodendrum infortunatum*, the range of zone of inhibition was observed as 0.2, 0.3, 0.3 (*Bacillus cereus*, *E.coli*) to 0.4cm. (*Klebsiella* sp. and *S.aureus*).



In case of chloroform extract of *Clerodendrum infortunatum* the range of zone of inhibition was observed as 0.2, 0.3 and 0.3 (Serratiasp., *E.coli* sp. and *Acinetobacter* sp.) to 0.4cm. (*Klebsiella* sp. and *Enterobacter* sp.) while about *Acinetobacter* sp. and *S.aureus* shows 0.5cm. The different range of inhibition zone was obtained which indicates the potential of plant extracts against selected bacterial strains (Fig.no. 4 and table no.4).

IV. CONCLUSION

The present study demonstrated that *Clerodendrum infortunatum* L. is a promising medicinal plant with considerable phytochemical and antimicrobial potential. The traditional use of *C. infortunatum* in the management of wounds and bone-related ailments, together with the phytochemical and antimicrobial findings of the present investigation, provides a scientific basis for further pharmacological exploration.

Qualitative phytochemical screening of the leaf extract revealed the presence of several bioactive constituents, including amino acids, phenolic compounds, flavonoids, coumarins, terpenoids, triterpenoids, anthraquinones, and steroids. The occurrence of these diverse classes of secondary metabolites may contribute to the pharmacological properties of the plant.

The antimicrobial evaluation further indicated that the leaf extract of *C. infortunatum* exhibited appreciable inhibitory activity against the selected bacterial strains, even at comparatively low concentrations. These findings suggest that the biologically active constituents present in the leaves may possess potential therapeutic relevance, particularly in relation to microbial control and the wound-healing process.

Therefore, *Clerodendrum infortunatum* L. may be considered a valuable plant for future research and development of plant-based therapeutic agents. Further needs to isolation and characterization of individual bioactive compounds, quantitative phytochemical analysis.

Table 1: Organoleptic properties of powder from selected plant species

Sr. No.	Name of Plants	Appearance	Colour	Smell	Taste
1.	<i>Clerodendrum infortunatum</i> L.	Powder	Green	Odour	Bitter

Table no.-2 : Physicochemical parameters

Sr.no.	Parameters	<i>Clerodendrum infortunatum</i> L.		
A.	Ash value (% w/w)			
1	Total ash	11.99		
2	Acid insoluble ash	3.99		
B.	Extractive value (% w/w)			
1.	Water soluble extractive	22.30		
2	Alcohol soluble extractive	24.37		
C.	Moisture content / Loss on drying (% w/w)	8.05		
D.	Foreign materials (% w/w)	1.91	1.91	1.91

Table no.3. Detection of Bioactive compound through Thin layer chromatography

Sr. No	Compounds	Band colour	RF value	Total no. of bands
1.	Phenols	Blue	0.05, 0.23, 0.80, 0.97	04
2.	Flavonoids	Yellow	0.61, 0.88	02
3.	Anthroquinone and anthracene	Yellow and red	0.10, 0.14, 0.54, 0.74, 0.88	05
4.	Coumarins	yellow blue and Blue	0.73, 0.78	02
5.	Diterpenoids	--	0.11, 0.50, 0.75, 0.94	04
6.	Triterpenoids	--	0.13, 0.98	02



7.	Steroids	--	0.25, 0.90	02
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Table no.4 : Antibacterial activity of Methanol and Chloroform extract of selected plant species

Sr.no.	Organisms	Zone of inhibition in cm.			
		Methanol extract	Chloroform extract	CON-1	CON-2
1	Acenetobactersp.	0.3cm	0.5cm	2.8cm	1.7cm
2	Klebsiellasp.	0.4cm	0.4cm	1.3cm	0.3 cm
3	S.aureus	0.4cm	0.5cm	2.2cm	1.5cm
4	E.coli.	0.3cm	0.3cm	2.5cm	1.5cm
5	Enterobactersp.	0	0.4cm	2.9cm	1.7cm
6	Bacillus cereus	0.2cm	0	2.7cm	2.6cm
7	Serratiasp.	0	0.2cm	2.3cm	1.8cm

CON 1-**Streptomycin** (+ Control), CON 2- **Gentamycin** (+ Control)

REFERENCES

1. Acharya, N. S. and Patel, J. J. (2016). Quality assessment and phytochemical analysis of *Clerodendrum serratum* roots. *International Journal of Phytopharmacy*, 6(3):51-57.
2. Arokiyaraj, S.; Sripriya, N.; Bhagya, R.; Radhika, B.; Prameela, L. and Udayaprakash, N.K. (2012). Phytochemical screening, antibacterial and free radical scavenging effects of *Artemisia nilagirica*, *Mimosa pudica* and *Clerodendrum siphonanthus* an invitro study. *Asian Pac J Trop Biomed*. 2:S601-S604.
3. Bennet, R. G. (1988). *Fundamentals of cutaneous surgery*. St. Louis C.V Mosby. 78.
4. Bray, W. J. (1977). The processing of leaf proteins to obtain food-grade products. In: "Green Crop Fractionation", Wilkins, R. J. (Ed.). Br. Grass. Soc. Occas. Publ. No. 9, Hurley, 107-116.
5. Daniel, M. (1991). *Methods in Plant Chemistry and Economic Botany*, Kalyani Publishers, New Delhi.
6. Dhal, P.; Dash, P.K.; Rout, J.R.; Srivastava, S.; Rath, C.C. and Sahoo, S.L. (2018). Phytochemical Analysis and Antibacterial Activity of *Clerodendrum philippinum* Schauer. *International Journal of Pharmacognosy and Phytochemical Research*, 10(4); 170-175.
7. Egger, K. (1969). Plant phenol derivatives, In: Stahl E (EDS) 1969. *Thin Layer Chromatography : A laboratory hand book*. J. Agric. Food Chem., 13(2):139-142.
8. Gouthamchandra, K.; Riaz, M.; Basheer, M.; Khadeer, A.; Parameshwarappa, S.B. and Venkatarangaiah, K. (2009). Wound healing activity of *Clerodendrum infortunatum* Linn root extracts. *Int. J. Biomed. Pharm. Sci.*, 3(1):21-5.
9. Harbone, J.B. (1998). *Phytochemical methods: A guide to Modern Technique of plant analysis*. Chapman and Hall, London.
10. Harborne, J. B. (1973). *Phytochemical Methods*. 1st ed. Chapman and Hall, London., 273.
11. Kaleab, A. and Adem, Y. (2013). Wound healing and antiinflammatory properties of *Allophylus abyssinicus* (Hochst.) Radlk. *Phytopharmacology*, 4(2):442-453.
12. Kalita, N and Kalita MC. (2014). Ethnomedicinal plants of Assam, India as an alternative source of future medicine for treatment of Pneumonia. *International Research Journal of Biological Sciences*, 3(10):76-82.
13. Kar, P.; Goyal, A.K.; Das, A.P. and Sen, A. (2014). Antioxidant and pharmaceutical potential of *Clerodendrum* L.: An overview. *International Journal of Green Pharmacy*, 210-216.
14. Katewa, S. S.; Chaudhary, B. L. and Jain Anita, (2004). Folk herbal medicines from tribal area of Rajasthan, India. *Journal of Ethnopharmacology*, 92 : 41-46.



15. Khandelwal, K. R. and Sethi, V. (2012). Practical Pharmacognocny: 2nd Edi. NiraliPrakashan, Pune.
16. Kokate, C. K.; Purohit, A. P. and Gokhale, S. B.(2002). Text book of Pharmacognosy. 18thedi. NiraliPrakashan Pune.
17. Korpenwar, A. N. (2012). Ethnomedicinal Plant ClerodendrumInfortunatumLinn. Used By The Traditional Practitioners For TheTreatment Of Cuts, Wounds And Burns. International Journal of Innovations in Bio-Sciences, 2 (1): 55-58.
18. Mbata, T. I.; Debiao, L. U. and Saikia, A.(2006). Antibacterial activity of the crude extract of Chinese green tea (Camellia sinensis) on Listeria monocytogenes. African Journal of Biotechnology. 7:1571-1573.
19. Norrel, S. A. and Messley, K.E. (1997). Microbiology Laboratory Manual Principles and Applications.” Prentice Hall, Upper Saddle River. New Jersey.,85-90.
20. Omale, J. and Victoria, I.A. (2010). Excision And Incision WoundHealing Potential OfSabaflorida(Benth) Leaf Extract In RattusNovergicus. International Journal on Pharmaceutical and Biomedical Research, 1(4):101-107.
21. Panthong, D.; Kanjanapothi, T.; Taesotikul, T. and Wongcomea, V. (2003). Anti-inflammatory and antipyretic properties of Clerodendrum petasites S. Moorea. Journal of Ethnopharmacology, 85: 151-156.
22. Patil, S. G.; Wagh, A. S.; Pawara, R. C. and Ambore S. M. (2013). Standard Tools for Evaluation of Herbal Drugs: An Overview. The Pharma Innovation- Journal, 2(9).
23. PrashithKekuda, T.R.; Dhanya Shree, V.S.; SaemaNoorain, G.K.; Sahana, B.K.; and Raghavendra, H.L. (2019). Ethnobotanical uses, phytochemistry and pharmacological activities of Clerodendrum infortunatum L. (Lamiaceae): A review. Journal of Drug Delivery & Therapeutics. 9(2):547-559.
24. Punekar, S. A. and Lakshminarasimhan, P. (2011). Flora of Anshi national park: Western Ghats-Karnataka. Biospheres Publication, Pune, India. 370.
25. Sannigrahi, S.; Mazumder, U. K.; Pal, D. and Parida, S. (2009). In vitro anti oxidant activity of methanolic extract of Clerodendrum infortunatum Linn. O.P.E.M., 9(2):128-34.
26. Santhosh, A. M.; Sravanthi, V.; Sri, Jhansi, U.; SanthiPriya, N. and Rama Rao, N. (2015). An overview of herbal possessing wound healing activity. e.j.p.m.r., 2(7):329-332.
27. Sharma, M. and Gupta, A. (2013). Preliminary phyto-chemical investigations of methanolic roots extract of Clerodendrum serratum: Antiulcer activity and histopathological study of stomach mucosa of wistar rats in ethanol induced ulc. Journal of Pharmaceutical Research and Opinion, 1 (7):202 – 208.
28. Shetty, B.V. ;Kaveriappa, K. M. and Bhat, G. K. (2002). Plant resources of Western Ghats and lowlands of Dakshina Kannada and Udupi districts. Pilikula Nisarga Dhama Society, Mangalore, India. 159.
29. Shukla, N.; Panda, C.S. and Mishra, S.K. (2015). Pharmacognostical Evaluation of Leaves of Two Medicinally Important Species of Clerodendrum. IJPRS, 4(3).
30. Singh, M. K.; Khare, G.; Iyer, S. K.; Shavan, G. and Tripathi, D.k. (2012). Clerodendrum serratum: A clinical approach. J. App. Pharma. Sci., 2(2):11-15.
31. Stahl, E. (1969). Thin Layer-Chromatography: A Laboratory handbook, Springer-Verlag Publication, Berlin.
32. Stevens, P. J. (1968). Thin-Layer Chromatography of synthetic steroids on silica gel impregnated with silver nitrate. J. Chromatography, 36: 253-258.
33. Surendrakumar, P. (1988). Anti-inflammatory activity of Lippianodiflora, Clerodendron phlomis and Delonix elata. Journal of Research Education Indian Medicine, 7: 19-20.
34. Tambe, V. D. and Bhambar, R. S. (2014). Estimation of total phenol, tannin, alkaloid and flavonoids in Hibiscus tiliaceus L. Wood extracts. Research and Review; Journal of pharmacognocny and phytochemistry. 2(4).
35. Taylor, J. L. S ;Rabe, T.; McGaw, L.J.; Jäger, A.K. and Staden, J. (2001). Towards the scientific validation of traditional medicinal plants. Plant Growth Regulation, 34: 23-37.



36. The Ayurvedic Pharmacopoeia of India. (2008). Government of India Ministry of Health & Family Welfare Department Of Ayush New Delhi. 1(3).
37. Thimmaiah, S. R. (1999). Standard Methods of Biochemical Analysis. Kalyani Publishers, New Delhi, 290-292.
38. Vidyai, S. M.; Nagori, B. P.; Gupta, R. N. and Bhatnagar, S. N. (2005). Evaluation of Anti-inflammatory, Analgesic and Anti-cancer Activity of Stem Bark Extracts of Prosopiscinerararia. Indian J. Nat. Prod., 21: 35-39.
39. Wagner, H. and Bladt, S. (1996). Plant drug analysis: A Thin Layer Chromatography, Atlas, 2nd eds. Springer-Verlag Publication, Berlin, Germany.
40. Wang, J.H.; Luan, E.; He, X.D.; Wang, Y. and Li, M-X. (2018) Traditional uses and pharmacological properties of Clerodendrum phytochemicals. Journal of Traditional and Complementary Medicine, 8: 24-38.

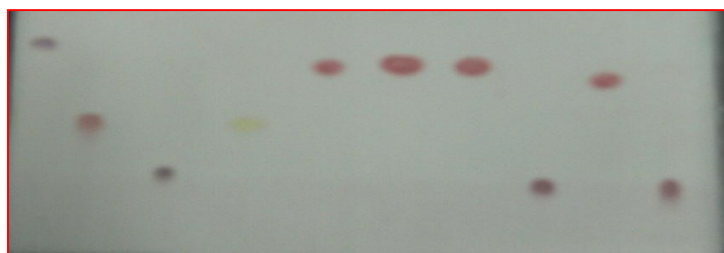


Fig: 1

: *Clerodendrum infortunatum* L



Glut Gly. Hist. Hydro. proli. Iso-leu nor- leu. Leu. Lyc. Meth A1 A2
Fig: 2 : Detection of standard Amino acids and Detection of free and bound Amino acids
(A1 & A2) from *Clerodendrum infortunatum* L Leaves of
Pseuderanthemum autropurpureum (W. Bull)



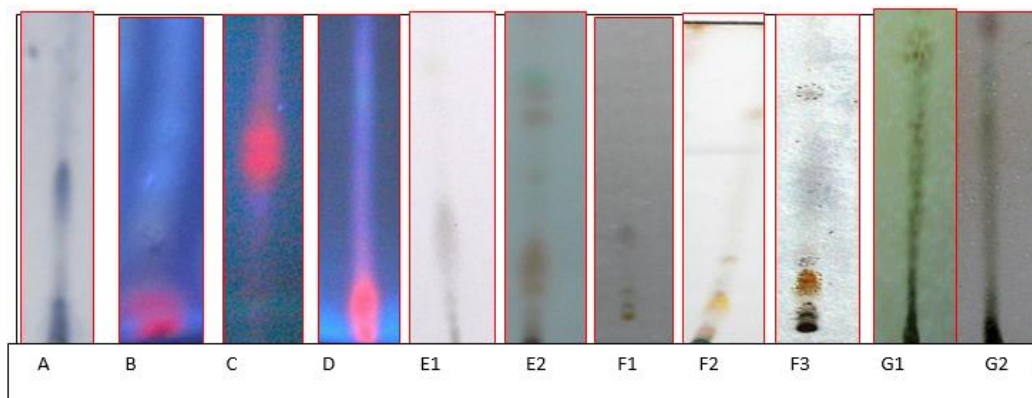


Fig: 3:- Detection of Secondary metabolites :-A: Phenol B: Flavonoid C: Anthroquinone D: Coumarins E1: Diterpenoids E2:Diterpenoids F1: Triiterpenoids F2: Triiterpenoids F3: Triiterpenoids G1:Steroids G2:Steroids

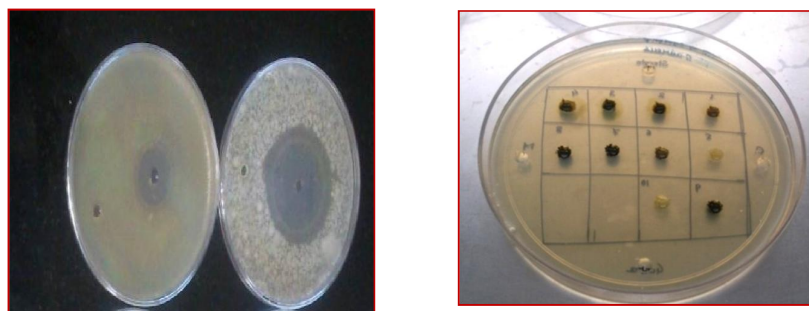


Fig: 4:-Inhibition zone of plant extract against bacterial species

