IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

ANTIMICROBIAL ACTIVITY ASSAY BY TLC BIOAUTOGRAPHY OF ETHYL ACETATE EXTRACT FROM THE LEAVES OF *WEDILIA TRILOBATA*AGAINST PATHOGENIC BACTERIA

[1]MANJULA M, [2*]PRAKASH K, [3]BAMA P and [4]SANKARANARAYANAN S

[1]Research Scholar, Department of Botany, Arignar Anna Government Arts College for Women, Walajapet- 632 513

[2]Assistant Professor, PG and Research Department of Botany, Arignar Anna Government Arts College, Villupuram-605602

[3]Professor, Department of Biochemistry, Sri Sairam Siddha Medical College and Research centre, Poonthadalam, West Tambaram, Chennai-600 048.

[4]Assistant Professor, Department of Medicinal Botany, Government Siddha Medical College, Arumbakkam, Chennai-600 106.

ABSTRACT

In present days, a search is going on for new antimicrobial agents due to the negative effects of existing antibiotics and the constant development of bacterial resistance. This study assessed the phyto chemical screening and antibacterial activities of methanol extract from the leaves of *Wedilia trilobata.* Methanol extract from the leaves of *Wedilia trilobata* has an antimicrobialactivities against 5 bacteria *K. pneumoniae, E. coli, E. faecalis, S. aureus* and *P. Aeruginosa* and it was investigated using a disc diffusion and minimum inhibitory concentration assay (MIC). Both disc diffusion and (MIC) of the extracts ranged from 5 to 20 µg/m. Results of phyto chemical screening of methanol extracts of *W. trilobata* showed the presence of tannins, flavonoids, terpenoids, alkaloid, poly phenols, saponins and cardiac glycosides absent. The total content of flavonoids and poly phenols in the

methanol extracts of the studied species positively correlated with their antioxidant properties .The study findings indicate that bioactive natural products from these plants may be isolated for further testing as leads in the development of new pharmaceuticals in food preservation as well as natural plant-based medicine.

Key words: *Wedilia trilobata*, antibacterial,pathogenic.TLC Bioautography

1.INTRODUCTION

In olden days, an imposing number of modern drugs have been isolated from natural sources. Most of the medicinal agents were obtained from the nature for thousands of years; most of them were based on the uses of the agents in the traditional medicines. 80% of the world's inhabitants depending predominantly on traditional system of medicines for their primary health care. Hence, the traditional system of medicines based on natural plants continues to play a major role in the health care. According to the World Health Organization, naturally occurring medicinal plants would be the best source to obtain a large number of drugs. Therefore, those plants should be taken under research to gain knowledge about their properties, safety and efficacy. In developing countries, there is a major cause of morbidity and reduced mortality due to the infectious diseases. Today, a vast range of synthetic and semi-synthetic antibacterial agents is available for the control of microorganisms; borne pathogenic microorganisms (Qi, 2015). *Wedilia trilobata*is a perennial herb with a creeping or climbing habit. The leaves are attractive, bright shiny green, somewhat fleshy oppositely arranged and simple, the blade obovate to elliptic or ovate and are stalk less. The single attractive bright yellow flower heads are daisy-like in appearance. The fruit is a 2 to 4-angled achene, with short, narrow pappus scales on the top .The aerial parts of this plant are used in traditional medicine in India used against bronchitis, colds, abdominal pains, dysmenorrheal, and even as a fertility enhancer. Also other folk medicine, it is employed to treat backache, muscle cramps, rheumatism, stubborn wounds, sores and swellings, and arthritic painful joints. The leaves used for treatment of kidney functioning, cold, stingray wounds, snakebite, purge and amenorrhea. However, the effect of methanol leaves extract of *Wedilia trilobata* on antibacterial activity have not been studied. The purpose of this study is to evaluate the antibacterial activity of methanol leaves extract of *Wedilia trilobata*.

2.MATERIALS AND METHODS

2.1. Plant material: The leaves of *Wedilia trilobata* was collected from Government siddha medical college, Arumbakkam, Chennai-600 106, Tamilnadu, India. The botanical identification of the plants was done by Dr. Sankaranarayanan, Head, Department of Medicinal Botany. A voucher specimen (GSMC-MB/215 and GSMC-MB/2016) was deposited in the herbarium of Department of Medicinal Botany. These plant materials were air-dried at room temperature and powdered. Then 500 g of each powder were macerated in methanol (2.5 l) at room temperature for 48 h. The filtrate was then concentrated under vacuum to give crude extracts from leaves of *Wedilia trilobata*. The extracts were stored at room temperature till further use.

2.2. Phyto chemical Analysis: Phyto chemical analysis was carried out by using the standard procedures to identify the constituents qualitatively in plant extracts, fractions and quantitatively in dried whole plant as described by Edeoga *et al.*

2.3. Bacterial strains: Antibacterial activities were conducted by Disc Diffusion method and Minimal Inhibitory Concentration Test (MIC) for all the selected plants against pathogenic bacteria (Gram positive and Gram negative). Bacteria used for the determination of antibacterial activities were Gram positive viz; *Staphylococcus aureus* MTCC 29213, *Klebsiellapneumoniae* MTCC 1771 and *Enterococcus faecalis* MTCC 439 and gram negative viz; *Pseudomonas aeruginosa* MTCC 2488, and *Escherichia coli* MTCC 25922.

2.4. Disc diffusion method: The agar disc diffusion method (NCCLS, 1997) was used for determinationof diameters of inhibition zones made by ethyl acetate extract from the leaves of *W.trilobatan* against various bacterial (*K. pneumoniae, E. coli, E. faecalis, S. aureus* and *P. aeruginosa*). Sterile nutrient agar was inoculated with 100 mL suspension of tested bacteria. The inoculated nutrient agar and potato dextrose agar were then poured into sterilized petri plates individually. Sterile filter discs impregnated with50 mL of sample solution were placed in inoculated petri plates using sterile forceps. Streptomycin was used as positive control in bacterial inoculated plates, respectively. The plates were incubated at 37 °C for 24 hours and at 27 °C for 48 hours for maximum bacterial growth, respectively. Antibacterial activities were evaluated by measuring diameter (mm) of inhibition zones using a zone reader.

2.5. Minimum inhibitory concentrations (MIC): Minimum inhibitory concentrations (MIC) were calculated by a modification of the reported method of Sarker *et al.*. For the evaluation of minimum inhibitory concentrations (MIC), different concentrations of plant extract, fractions and essential oil were prepared by serial dilution. The range of dilution was determined by keeping in mind the antimicrobial activity determined in the inhibition zone assay. For the samples showing better activity in the first assay the serial dilution for MIC determination was carried out at less concentration and for the samples showing less activity the higher concentration was used for serial dilution. Ciprofloxacin and fun gone at 10µg/mL were used as reference standards for the bacterial and fungal strains, respectively.

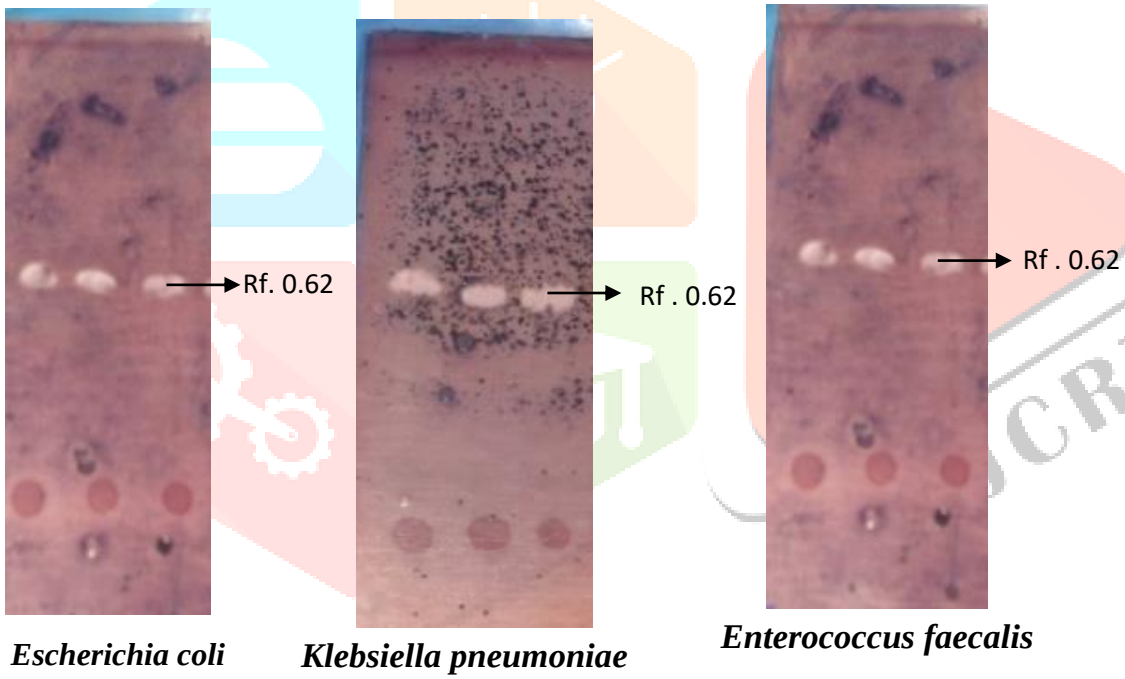
2.6.Statistical analysis: Minitab software version 16 (Statistical software, Minitab Inc.,State College, PA, USA)was used to perform analysis of variance (ANOVA) and to determine significant differences ($p < 0.05$).

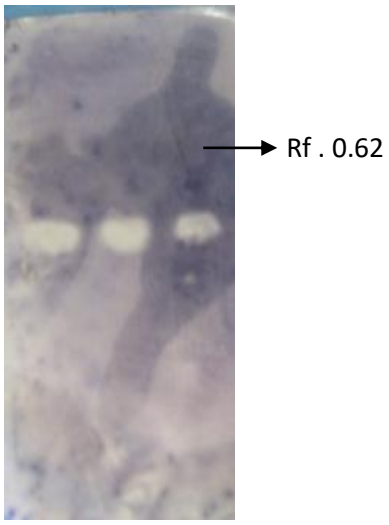
3. RESULT AND DISCUSSION

The areas of inhibition (colored white/light yellow on a purple/pink background) were compared with the Rf value of the related spot on the reference plate. Active compounds against *Klebsiella pneumoniae, Escherichia coli, Enterococcus faecalis, Staphylococcus aureus,* and *Pseudomonas aeruginosa* were contained in an ethyl acetate extract from the leaves of *Wediliatrilobata* with varying Rf values of 0.62. Other phytochemical extracts have shown to have little effect on microbial growth in some cases. Straight bioautography combined with thin layer chromatographic (TLC) separation is a fast and responsive antimicrobial compound screening process. Testmicroorganism cultures are accomplished of growing unswervingly on theTLC plate, so each step of the assay is achieved on the sorbent (Fig-1).TLC has been efficient technique for separate bioactive compounds from crude extract (Bucar et al., 2013). Similarly, Syahriel Abdullahet al., (2021) reported that antibacterial compound isolation from *Ganoderma boninense*(Rf = 0.4) was tested both gram positive and gram negative bacteria. The ethyl acetate extract of *Wedilia trilobata* bioautography result confirmed that the compounds revealed in TLC have antibacterial activity against the bacteria tested. These results are in agreement with other studies on chromatography and bioautography reported previously Chen and Schwack, 2014 and another report also established which bioactive compounds were found in fungal extracts of Terminalia arjuna (Gill and Vasundhara, 2019).

The ethyl acetate extract from the leaves of *Wediliatrilobata* had the highest flavonoid and terpenoid content, as well as the best antibacterial activity.The chemical composition that can lead to this behavior is determined using GC-MS analysis.The GC-MS study revealed a number of different phenolic compounds (Table-2 and Fig-2). Furthermore, due to their strong free radical scavenging properties, many cinnamic acid derivatives with the phenolic hydroxyl group is considered antidiabetic and are expected to have many health benefits. Analysis carried out by GC-MS of the methanolic extracts of whole plant (present study) revealed presence of 11 compounds at molecular level viz. Oleic Acid, Phytol, n-hexadecanoic Acid, 9-Octadecenoic acid(Z)-, methyl ester. All these bioactive compounds are responsible for the antibacterial property and other therapeutic uses (Mishra et al., 2014)

Fig-1. Antimicrobial activity assay by TLC bioautography of ethyl acetate extract from the leaves of *Wediliatrilobata*against pathogenic bacteria





Pseudomonas aeruginosa

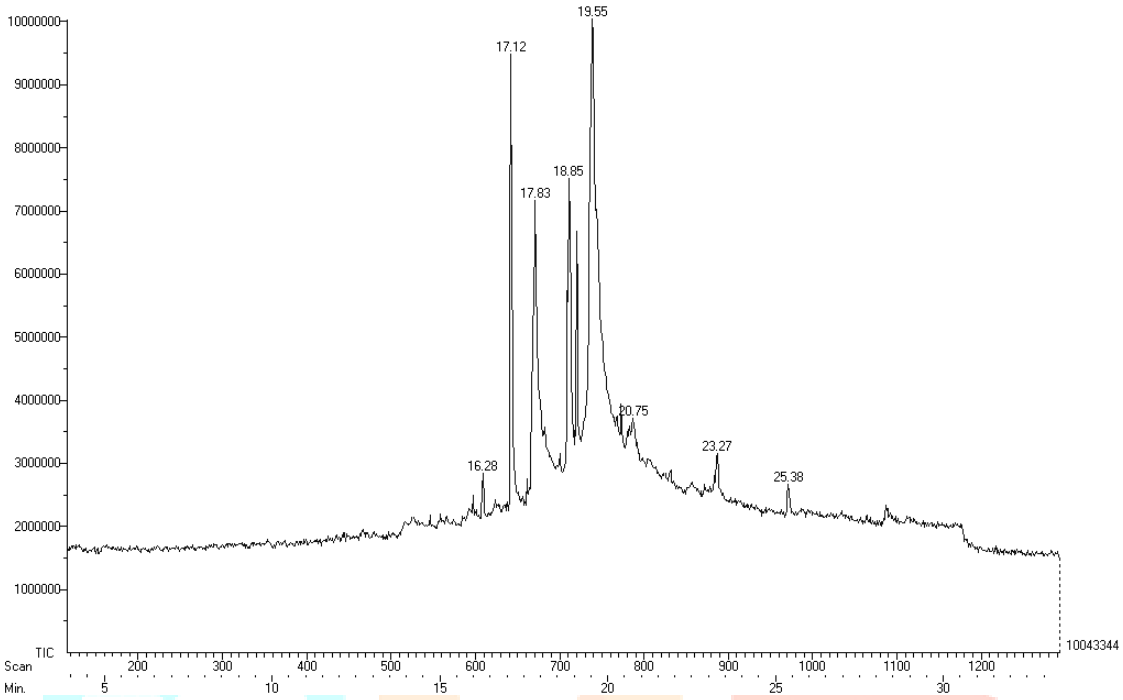
Staphylococcus aureus

Black arrow indicates Rf value 0.62 – suppressed Pathogenic Bacterial growth

Table-2 GC–MS analysis of ethyl acetate extract from the leaves of *Wediliatrilobata*

S.No	Compound	Retention Time(min)	Molecular weight	Major peaks
1	Pentadecanoic acid, 14-methyl-, methyl ester	17.2	167	240
2	3-Methylene-4-phenyltricyclo	17.8	143	227, 270
3	Tricyclo [7.2.2.0(3,8)] tridec-12-en-2-one, 5,6-epoxy-4-methyl	18.35	167	224
4	Tricyclododec carboxy ethoxy	19.65	117	143, 181
5	*Benzamide, 2-amino-5-hydroxy*	18.9	136	198, 228
6	Eicosatetraenoic acid	20.48	165	207, 253
7	Hexadec-9-enoic acid	22.37	111	236, 254

Fig-2. GC–MSConsolidated retention Timeofethyl acetate extract from the leaves of *Wediliatrilobata*



CONCLUSION

In the present study results indicate that the ethyl acetate extract of *W. trilobata* possess antimicrobial, properties. The ethyl acetate extract of this plant showed strong antibacterial activity compared standard antibiotic. These reports provide a basic scientific evidence to support its traditional medicinal uses. In this study might suggest a possible use of *W. trilobata* as source of natural antibacterial, agent. The ethyl acetate extract of *Wediliatrilobata* had the highest antibacterial activity against *E. coli, K. pneumoniae, P. aeruginosa, E. faecalis*, and *S. aureus*. TLC analysis revealed five UV fluorescent compounds in the ethyl acetate extract of *Wediliatrilobata* leaves.The bioautography of TLC plates of *Wediliatrilobata* ethyl acetate extract with Rfvalue 0.62 revealed an area that inhibited pathogenic bacteria development. Seven different phenolic compounds were discovered using GC-MS. The phenolic hydroxyl group was thought to be antibacterial in several Phenol 2,6-dimethoxy-derivatives.

REFERENCE

1. Bucar, F., Wube, A., and Schmid, M. 2013. Natural product isolation–how to get from biological material to pure compounds. Nat. Prod. Rep. 30, 525–545.

2. Chen Y, Schwack W (2014). High-performance thin-layer chromatography screening of multi class antibiotics in animal food by bioluminescent bioautography and electrospray ionization mass spectrometry. Journal of Chromatography A 1356:249-257.

3. Edeoga, H.O., Okwu, D.E., Mbaebie, B.O. 2005. Phytochemical constituents of some Nigerian medicinal plants. *J. Biotechnol.,4*, 685–688.

4. Gill H, Vasundhara M (2019). Isolation of taxol producing endophytic fungus Alternaria brassicicola from non-Taxus medicinal plant Terminalia arjuna. World Journal of Microbiology and Biotechnology 35(5). doi:10.1007/s11274-019-2651-8.

5. Mishra P, Jamdar P, Desai S, Patel D, Meshram D. Phytochemical analysis and assessment of in vitro antibacterial activity of Tinospora cordifolia. International Journal of Current Microbiology and Applied Sciences; 3(3): 224- 234 (2014).

6. NCCLS (National Committee for Clinical Laboratory Standards). Performance standards for antimicrobial disc susceptibility test. Approved Standard. 6th ed. Wayne, PA:NCCLS; 1997. M2eA6.

7. Qi, Z., 2015. WHO Traditional Medicine Strategy 2014-2023: Background and progress in the last decade. Glob. Heal. Hist. Semin. Tradit. Med. Ayurveda 1–28.

8. Syahriel A, Yoon Sin O, Min-Kyu K and KhimPhin C. (2021). Biophysical characterization of antibacterial compounds derived from pathogenic fungi Ganoderma boninense Journal of Microbiology, 59 (2), 164–174.