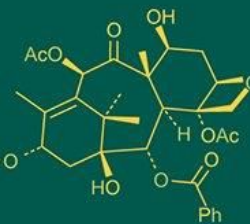
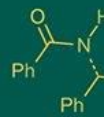


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; SP-8(7): 942-946
www.biochemjournal.com
Received: 09-06-2024
Accepted: 11-07-2024

Pratima Raypa

Department of Biochemistry,
College of Basic Sciences and
Humanities, G.B. Pant Univ.
of Ag. & Tech., Pantnagar,
Uttarakhand, India

AK Verma

Department of Biochemistry,
College of Basic Sciences and
Humanities, G.B. Pant Univ.
of Ag. & Tech., Pantnagar,
Uttarakhand, India

Salil Tewari

Department of Genetics and
Plant breeding, G. B. Pant
Univ. of Ag. & Tech.,
Pantnagar, Udham Singh
Nagar, Uttarakhand, India

Ashutosh Dubey

Department of Biochemistry,
College of Basic Sciences and
Humanities, G.B. Pant Univ.
of Ag. & Tech., Pantnagar,
Uttarakhand, India

Corresponding Author:**Pratima Raypa**

Department of Biochemistry,
College of Basic Sciences and
Humanities, G.B. Pant Univ.
of Ag. & Tech., Pantnagar,
Uttarakhand, India

Study of the potential antibacterial activity of plant extracts of *Adina cordifolia* using the thiazolyl blue tetrazolium bromide (MTT) assay

Pratima Raypa, AK Verma, Salil Tewari and Ashutosh Dubey

DOI: <https://doi.org/10.33545/26174693.2024.v8.i7SI.2581>

Abstract

The therapeutic effects of plant extracts, which have high activity against a number of ailments because they contain helpful bioactive components, are gaining more and more attention. The antibacterial activity of *Adina cordifolia* extracts was studied. Thus, the purpose of this study was to assess the effectiveness of *Adina cordifolia* leaf and root extracts against possible bacterial strains using chloroform, ethyl acetate, acetone, methanol and aqueous extracts. Two bacterial strains, *Salmonella typhimurium* and *E. coli* were subjected for antibacterial activity. A good inhibitory and bactericidal action was demonstrated by *Adina cordifolia* extracts against bacterial strains because of the presence of unique bioactive components including 7-hydroxycoumarin.

Keywords: 7-hydroxycoumarin, MTT assay, *Adina cordifolia*, *Salmonella typhimurium*

1. Introduction

Numerous plants provide a variety of therapeutic compounds that are useful for both medical and agricultural purposes. Given that many people worldwide rely on traditional plant-based treatments due to the scarcity and high cost of contemporary medications, plants offer enormous promise as sources of medicinal and pharmacopoeial drugs [1]. Many herbal remedies that have been made possible by these medicinal plants are essential for the creation of novel medications that are used to cure and prevent illness [20].

The deciduous tree known as haldu (*Adina cordifolia*) is native to South Asia and can be found from eastern India and Sri Lanka to southern China and Vietnam. It is a member of the Rubiaceae family, subfamily Cinchonoideae. It thrives in regions as high as 900 meters above sea level south of the Himalayas and is dispersed over the majority of India's deciduous woods. According to www.fes.org.in, *A. cordifolia* is in danger of being extinct. Oriental medicine has long employed *A. cordifolia* as an essential ingredient in a variety of antiseptic and antipyretic medicines [5].

The bark is hot, bitter, and aromatic; it is used to cure liver problems and is strengthening, healing, and aphrodisiac in nature. According to Chadha (1985), the roots are used as an astringent for diarrhea. Due to its active ingredients' intriguing H⁺/K⁺-ATPase inhibitory actions, *A. cordifolia* was also investigated for its ability to treat ulcers [9]. According to Rokade and Pawar (2013), four chemicals that were recovered from a strain of *Adina cordifolia* were identified as stigmasta-5, α -amyrin, octacosanol, 22-diene-3P-O- α -rhamnopyranosyl-(1-4)-P-dixylopyranoside and naringenin-7-methyl ether-4'-O- α -rhamnopyranoside. The greatest technique for protecting the endangered *Adina cordifolia* in the wild and quickly multiplying plant populations is through apical bud *in vitro* propagation. According to Raypa *et al.* (2013), *Adina cordifolia* was highly well established *in vitro* when MS medium was added with either 0.5 mg/L NAA or 2 mg/L BAP. The main compounds identified in the leaf and bark extracts of *Mitragyna parvifolia* (Rubiaceae) were butyric acid, 2-ethylhexyl ester (19.36%), 4-methylmannose (53.13%), mitraphylline (21.59%), isomitraphylline (3.37%), 1,2-hydrazinedicarboxylic acid, diethyl ester (3.50%), α , α -dimethylmuconic acid (22.97%), isobutanoic acid, 2-ethylhexyl ester (43.83%), α -D-glucopyranoside, α -D-glucopyranosyl (27.21%).

M. parvifolia leaves include compounds such as the pentacyclic oxindole alkaloid mitraphylline, which has been shown to have anti-inflammatory and anti-proliferative qualities [22].

2. Materials and Methods

2.1. Materials

The leaves and roots of *Adina cordifolia* were collected from Agro-forestry Research Centre (AFRC) Haldi (29.06441° N, 79.82281° E) G. B. Pant University of Agriculture and Technology, Pantnagar in the month of March.

2.2 Experimental work

2.2.1 Preparation of plant material

Leaves and roots were washed thoroughly and oven dried at 40 °C. Then dried samples were grounded to make powder in mechanical grinder and stored in air tight container.

2.2.2 Preparation of samples

Plant extracts were prepared in five different solvents viz chloroform, ethyl acetate, acetone, methanol and water. Powdered plant material was used for respective solvent (w:v) (1:4) extraction. The extracts were filtered; evaporated and dried respective extracts were used for experimentation.

2.2.3 Statistical analysis

All the analyses were performed in triplicates and results were reported as means $\pm$ standard deviation (SD). The significance of differences among treatment means were determined by one way analysis of variance (ANOVA) with a significant level of ($p=0.05$).

2.3 Antibacterial assay in different leaf and root extracts against selected human pathogens

2.3.1 Test microorganisms and growth conditions

The animal pathogenic bacterial strains isolated from faecal matter. All bacterial strains were grown in nutrient agar (NA) plates at 37 °C. Glycerol stocks were also prepared for each culture and stored at -20 °C. Every culture was streaked on the NA plate each time before experiment and for cell viability assay each bacterial culture was grown on nutrient broth (NB).

Pathogenic bacterial strains

1. *Salmonella typhimurium* 52
2. *Escherichia coli* 13

2.3.2 Determination of antibacterial assay via MTT assay

The antibacterial assay was determined by MTT-tetrazolium-based colorimetric assay [13] with slight modifications. In this assay the reduction of the tetrazolium salt MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) to blue formazan product due to mitochondrial enzymes was measured spectrophotometrically. MTT dye of 5 mg/mL was prepared in phosphate saline buffer (1X). Each extract starting from 1 mg was serially diluted and volume was made up to 100 μ L with methanol in each well of a 96-well microplate. Last well was used as methanol blank without any extract in it. The bacterial cell suspension (100 μ L) was plated at a density of approximate 1.5×10^8 cells per well with help of Mc-Farland standard and incubation was done for 24h,

followed by adding 20 μ L (5 mg/mL) of MTT dye to each well. Excess MTT was discarded after one hour incubation at 37 °C and the intracellular purple insoluble formazan was solubilized by adding 50 μ L/well DMSO. After shaking on orbital shaker for 20 mins the optical density (OD) was measured at 570 nm in microplate reader. Gentamycin (1mg/mL) was used as a positive control prepared in phosphate saline buffer (1X) to calculate the cytotoxicity. All tests and analyses were run in triplicate and mean values were recorded. Phosphate saline buffer (1X, g/L) was prepared in desired volume containing CaCl_2 anhydrous (0.10 g), KCl (0.20 g), KH_2PO_4 (0.20 g), $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (0.10 g), NaCl (8.00 g), Na_2HPO_4 (1.15 g) anhydrous and the pH was adjusted at 7.4.

In the present study, leaf and root extracts (obtained in chloroform, ethyl acetate, acetone, methanol and water) of *Adina cordifolia* were assayed to check cell viability of six gram negative human pathogenic strains i.e., *Salmonella typhimurium* 52, *Salmonella typhimurium* 149, *Salmonella typhimurium* ATCC 51812, *Salmonella enteritidis* ATCC 13076, *E. coli* ATCC-25922, and *E. coli* strain-13 by the method of Mosmann (1983) using gentamycin as a positive control with some modification. Cell viability was determined by using tetrazolium salt, MTT (3-[4, 5-dimethylthiazolyl-2]-2,5-diphenyltetrazolium bromide) based colorimetric assay. MTT is the commonly applied method for evaluation of cell viability and cytotoxicity for screening the drugs. The MTT assay based on the reduction of MTT (yellow colored) depends upon cellular metabolic activities due to NAD (P)H-dependent cellular oxidoreductase enzymes [3]. This method uses colorimeter to determine the cell viability [16]. In MTT assay, the linear relationship between metabolically active cells and the colour produced is established, thus allowing an accurate quantification of changes in the rate of cell death or proliferation [3].

3. Results

3.1 Antibacterial assay for cytotoxicity of various extracts of leaf and root of *Adina cordifolia* against *Salmonella typhaemurium* 52

Salmonella typhaemurium is a gram negative bacteria causing gastroenteritis, poisoning and typhoid fever [10]. The increasing effectiveness of plant extracts were determined on the basis of viability percentage of the cells. The extracts concentration were 62.5 μ g/mL, 125 μ g/mL, 250 μ g/mL, 500 μ g/mL and 1000 μ g/mL. Cell viability percentage decrease was observed as the concentration of plant extracts were increased.

Salmonella typhaemurium 52 was treated with the all five (chloroform, ethyl acetate, acetone, methanol and water) leaf extracts of *Adina cordifolia* and antibiotic, gentamycin was taken as positive control. Among all the leaf extracts, acetone extract showed good cytotoxicity against *S. typhaemurium* 52. In leaf acetone extract, only 52.47% viable cells were found at 1000 μ g/mL concentration (i.e. mortality percentage was 47.53%). In leaf methanol, water, ethyl acetate and chloroform showed 61.24%, 64.99%, 65.34% and 69% viable cells respectively at 1000 μ g/mL concentration Fig.1) i.e. mortality percentage was 38.76%, 35.01%, 34.66% and 31% in leaf methanol, water, ethyl acetate and chloroform extracts respectively. Gentamycin showed 28.71% viable cells in 1000 μ g/mL concentration (i.e. mortality percentage was 71.29%). Single compound 7-

hydroxycoumarin which is key component in root (not found in leaf except only trace amount found in leaf water extract) showed 47% viable cells at 1000 $\mu\text{g/mL}$ concentration i.e. mortality percentage was 53%.

In root extracts of *Adina cordifolia*, root ethyl acetate extract showed maximum cytotoxicity against *S. typhimurium* 52. In root ethyl acetate extract, only 56.4% viable cells were found at 1000 $\mu\text{g/mL}$ concentration (Fig.2 (i.e. mortality percentage was 43.6%). In root extracts, chloroform, acetone, methanol and water showed 56.40%, 59.86%, 65.05% and 79.75% viable cells respectively at 1000 $\mu\text{g/mL}$

concentration i.e. mortality percentage was 43.6%, 40.14%, 34.95% and 20.25% in chloroform, acetone, methanol and water extracts respectively. The result showed clearly that different extracts of *Adina cordifolia* irrespective of the plant parts and solvent used, were able to inhibit bacterial growth to a significant level. Thus the use of plants like *Adina cordifolia* for prevention and cure of different bacterial ailments might reduce the dependence on synthetic drugs (antibiotics), as well as adverse effects associated with them.

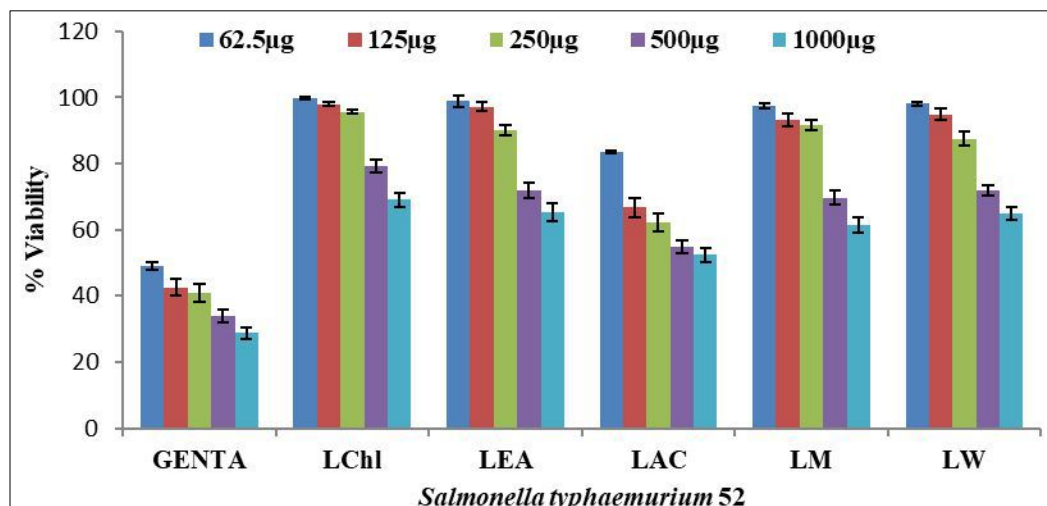


Fig 1: Viability Percentage of *Adina cordifolia* leaf chloroform, ethyl acetate, acetone, methanol and water extracts against *Salmonella typhimurium* 52

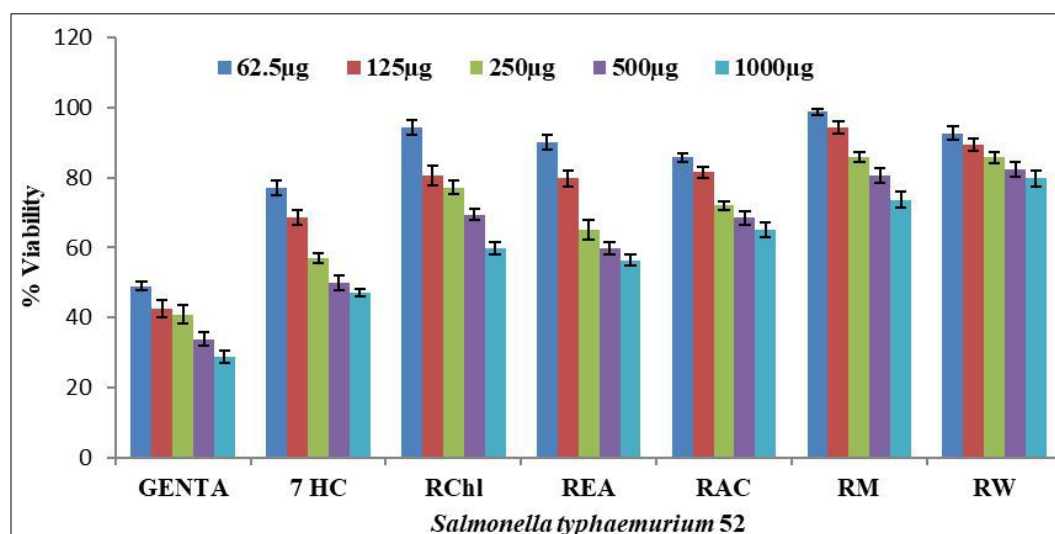


Fig 2: Viability Percentage of *Adina cordifolia* root chloroform, ethyl acetate, acetone, methanol and water extracts against *Salmonella typhimurium* 52

3.2 Antibacterial assay for cytotoxicity of various extracts of leaf and root of *Adina cordifolia* against *E. coli* strain-13

E. coli also reported to cause severe cases including bloody diarrhea, dehydration and kidney failure [6]. *E. coli* strain-13 was treated with the all five leaf extracts of *Adina cordifolia*. Among all the leaf extracts, water extract showed good cytotoxicity against *E. coli* strain-13. In leaf water extract, only 47.84% viable cells were found at 1000 $\mu\text{g/mL}$ concentration (i.e. 52.16% cells had been killed by extract). In leaf extracts, ethyl acetate, chloroform, acetone and methanol showed 54.5%, 56.34%, 60.70% and 63.23%

viable cells respectively at 1000 $\mu\text{g/mL}$ (Fig.3) i.e. mortality percentage was 45.5%, 43.66%, 39.3% and 36.77% in ethyl acetate, chloroform, acetone and methanol leaf extracts respectively. Antibiotic, gentamycin was taken as positive control. Gentamycin showed 28.9% viable cells in 1000 $\mu\text{g/mL}$ concentration i.e. gentamycin had highest mortality percentage (71.1%). Single compound 7-hydroxycoumarin which is key component in root showed 45.59% viable cells at 1000 $\mu\text{g/mL}$ concentration (i.e. 54.41% cells had been killed by compound).

In root extracts of *Adina cordifolia*, root ethyl acetate extract showed maximum cytotoxicity against *E. coli* strain-13. In

root ethyl acetate extract, only 45.07% viable cells were found at 1000 µg/mL concentration (i.e. 54.93% cells had been killed by extract). In root extracts, water, acetone, methanol and chloroform showed 47.72%, 48.67%, 49.42% and 69.35% viable cells respectively at 1000 µg/mL concentration (Fig. 4.) i.e. mortality percentage was 52.28%, 51.33%, 50.58% and 30.65% in water, acetone, methanol and chloroform root extracts respectively (Fig.3 and Fig. 4). The result showed clearly that different extracts of *Adina*

cordifolia irrespective of the plant parts and solvent used, were able to inhibit bacterial growth to a significant level. Diffusion methods (agar disk diffusion and agar well diffusion) have been widely used to screen the antimicrobial activity of extracts and their main compounds however, these tests do not permit the quantification of their bioactivity in terms of minimum inhibitory concentration (MIC), since they are qualitative tests ^[19].

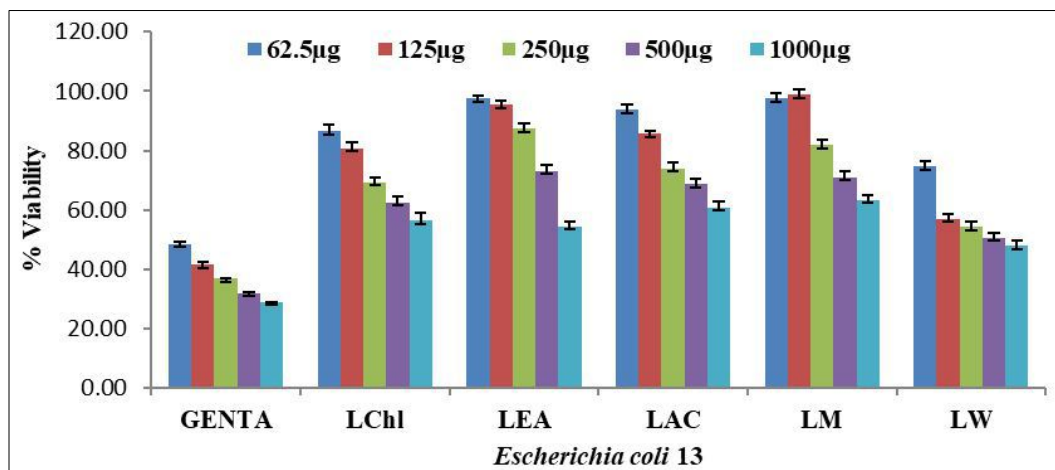


Fig 3: Viability Percentage of *Adina cordifolia* leaf chloroform, ethyl acetate, acetone, methanol and water extracts against *E. coli* strain-13.

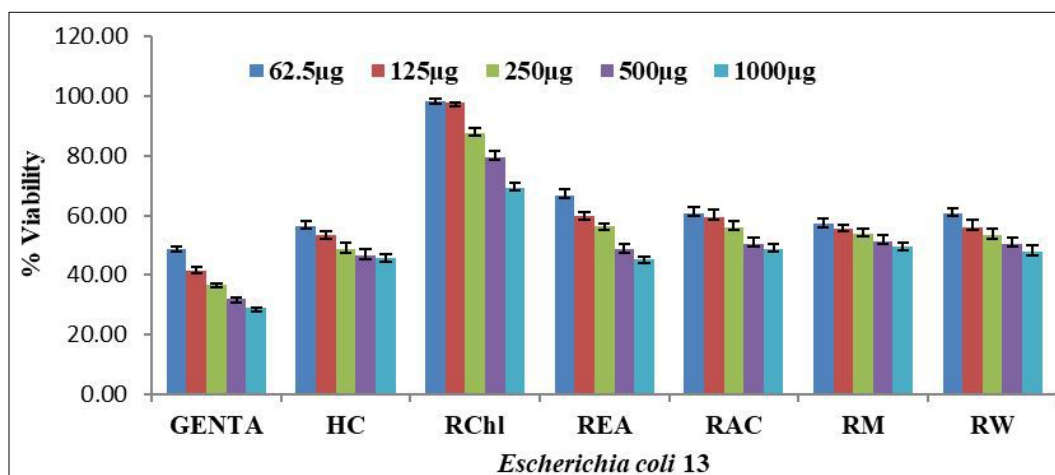


Fig 4: Viability Percentage of *Adina cordifolia* root chloroform, ethyl acetate, acetone, methanol and water extracts against *E. coli* strain-13.

GENTA= Gentamycin, HC= 7-Hydroxy coumarin, Rchl= Root chloroform extract, REA=Root ethyl acetate, RAC=Root acetone, RM=Root Methanol, RW=Root Water

4. Discussion

Antibacterial assay of 10 extracts of leaf and root of *Adina cordifolia* was evaluated against 2 gram negative human pathogenic strains i.e., *Salmonella typhimurium* 52 and *E. coli* strain-13 by using MTT assay. Single compound, 7-hydroxycoumarin which is potent metabolite of roots, had great antibacterial potential. Leaf and root extracts showed the anti microbial activity. Thus this plant extracts can be used as pharmacogenosic efficacy.

Maji *et al.*, (2010) reported the antimicrobial efficiency of 20 ethnomedicinal plants (crude leaf extracts) by using water, benzene and acetone as solvents and tested against seven human pathogens like *Escherichia coli* (MDR), *Staphylococcus aureus* (MDR), *Klebsiella pneumoniae*, *Bacillus cereus*, *Vibrio cholerae* and *Candida albicans*.

Dash *et al.*, (2014) evaluated *in-vitro* antibacterial activities of methanolic extract of *Haldina cordifolia* against a series of various bacterial strains by well diffusion method using nutrient agar medium. Antibacterial activities were found to be quite inspiring as it has shown inhibitory effect against all the pathogens like *Staphylococcus aureus* ATCC 29757, *Pseudomonas aeruginosa* ATCC 25619, *Escherichia coli* ATCC 10536, *Streptococcus pneumoniae*, *Clostridium difficile*, *Klebsiella pneumoniae*, *Mycobacterium tuberculosis* and *Bacillus subtilis* ATCC 10389. Antibacterial activity was determined by the well diffusion method using nutrient agar medium. Tetracycline was used as a positive control.

5. Conclusion

Adina cordifolia extracts possess unique bioactive components, such as 7-hydroxycoumarin, which have been shown to have good bactericidal and inhibitory effect against bacterial strains.

6. Acknowledgement

Authors are grateful to Dept. of Biochemistry, GBPUA&T, Pantnagar to carry out this research work. Our sincere thanks to DSTFIST for providing equipment facility and Directorate of Experiment Station (DES) Pantnagar for utilization of infrastructure.

7. Competing Interests

Authors have declared that no competing interests exist.

8. References

1. Aparna V, Dileep KV, Mandal PK, Karthe P, Sadasivan C, Haridas M. Anti-inflammatory property of n-hexadecanoic acid: structural evidence and kinetic assessment. *Chemical Biology & Drug Design*. 2012;80(3):434-439.
2. Asfaw A, Lulekal E, Bekele T, Debella A, Meresa A, Sisay B, *et al.* Antibacterial and phytochemical analysis of traditional medicinal plants: An alternative therapeutic approach to conventional antibiotics. *Heliyon*. 2023;9.
3. Berridge MV, Herst PM, Tan AS. Tetrazolium dyes as tools in cell biology: New insights into their cellular reduction. *Biotechnology Annual Review*. 2005;11:127-152.
4. Chanda S, Dave R. *In vitro* models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview. *African Journal of Microbiology Research*. 2009;3(13):981-996.
5. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian medicinal plants. *Planta Medica*. 1956;43:59-63.
6. Dalu AP, Zagare VS, Avchar PE, Kadam MP, Ingole AS, Nagrik SU, *et al.* A pharmacological potential of *Adina cordifolia*. *Journal of Drug Delivery and Therapeutics*. 2021;11(2-s):132-135.
7. Dash PP, Chaturvedi AK, Mishra A, Chaturvedi D. Search for an herbal medicine: Antibacterial activity of methanolic extract of *Haldina cordifolia*. *International Journal of Natural Products Research*. 2014;4(4):95-99.
8. Duke JA, Beckstrom-Sternberg SM. Dr. Duke's Phytochemical and Ethnobotanical Databases; c1994.
9. Fujita E, Saeki Y, Ochiart M, Inoue T. Investigation of the neutral constituents of *Lythrum salicaria* L. *Bulletin of the Institute for Chemical Research*. 1972;50:327-331.
10. Gart EV, Suchodolski JS, Welsh Jr TH, Alaniz RC, Randel RD, Lawhon SD. *Salmonella typhimurium* and multidirectional communication in the gut. *Frontiers in Microbiology*. 2016;7:1827.
11. Kanthel LK, Dey A, Satyavathi K, Bhojaraju P. GC-MS analysis of bio-active compounds in methanolic extract of *Lactuca runcinata* DC. *Pharmacognosy Research*. 2018;6:58-61.
12. Kasinadhuni VRR, Rajashekhar G, Rajagopalan R, Sharma VM, Vamsi Krishna C, Sairam P, Sai Prasad G, *et al.* Anti-ulcer potential of *Haldinia cordifolia*. *Fitoterapia*. 1999;70:93-95.
13. Levitz SM, Diamond RD. Mechanisms of resistance of *Aspergillus fumigatus* conidia to killing by neutrophils *in vitro*. *The Journal of Infectious Diseases*. 1985;152:33-42.
14. Lorget MD, Salen P, Laporte F, Leiris JD. Alpha-linolenic acid in the prevention and treatment of coronary heart disease. *European Heart Journal Supplements*. 2001;3:26-32.
15. Maji S, Dandapat P, Ojha D, Maity C, Halder SK, Mohapatra PKD, *et al.* *In vitro* antimicrobial potentialities of different solvent extracts of ethnomedicinal plants against clinically isolated human pathogens. *Journal of Phytology*. 2010;2:57-64.
16. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*. 1983;65:55-63.
17. Rathna N, Bakkiyara D, Shunmugiah P. Anti-biofilm mechanisms of 3, 5-di-tert-butylphenol against clinically relevant fungal pathogens. *Biofouling*. 2016;32(9):979-993.
18. Raypa P, Verma AK, Dubey A, Tewari SK. *In vitro* establishment of a threatened plant species *Adina cordifolia*. *Octa Journal of Biosciences*. 2013;1(2):138-142.
19. Requena R, Vargas M, Chiralt A. Study of the potential synergistic antibacterial activity of essential oil components using the thiazolyl blue tetrazolium bromide (MTT) assay. *LWT - Food Science and Technology*. 2019;101:183-190.
20. Rokade S, Pawar SP. A comprehensive review on *Adina cordifolia*. *International Journal of Pharmaceutical Sciences Review and Research*. 2013;18:13-16.
21. Van de Loosdrecht AA, Beelen RH, Ossenkoppele GJ, Broekhoven MG, Langenhuijsen MM. A tetrazolium-based colorimetric MTT assay to quantitate human monocyte-mediated cytotoxicity against leukemic cells from cell lines and patients with acute myeloid leukemia. *Journal of Immunological Methods*. 1994;174:311-320.
22. Vasmatkar P, Dubey A, Tyagi B, Baral P, Tandon S, Kadam A. Antibacterial activity and GC-MS analysis of methanolic extract from stem bark and leaves of *Mitragyna parvifolia* (Roxb.) Korth. *Indo American Journal of Pharmaceutical Research*. 2014;4(1):304-311.
23. Xu H, Lybrand D, Bennewitz S, Tissier A, Last RL, Pichersky E. Production of trans-chrysanthemic acid, the monoterpene acid moiety of natural pyrethrin insecticides, in tomato fruit. *Metabolic Engineering*. 2018;47:271-278.