https://doi.org/10.48047/AFJBS.6.Si4.2024.290-299

African Journal of Biological Sciences

Investigation On The Antimicrobial Potential Of Some Herbal Medicinal Plants Used In Folk Medicine From Uttarakhand, India

Monika Basotra[1], Anuradha Jayaraman[2]*, Madhvi Sharma[3], Rakhi R. Maurya[4] and Sadhana Rai[5]

[1,2*,3,4,5]Department of Botany, Nims Institute of Allied Medical Science and Technology, NIMS University (Rajasthan) Jaipur-303121.

***Corresponding author:-** Anuradha Jayaraman
Email ID: j.anuradha@nimsuniversity.org

Article Info
Volume 6, Issue Si4, 2024
Received: 25 April 2024
Accepted: 01 June 2024
doi: 10.48047/AFJBS.6.Si4.2024.290-299

Abstract:
Microbial infections are causing life threatening diseases which may be treated by use of antibiotic drugs. In few years, prevention of such infections has become challenging due to enhancing development of microbial resistance against currently using antibiotic drug. So, there is need for development of new antimicrobial drugs. Chemically synthetic compounds are more expensive and have more side effects. Discovery of natural antimicrobial compounds from plants may solve the problem. In the present study, ethanolic extracts of leaves of selected 5 plants *Viz Artemisia annua, Artemisia vulgare, Picrorhiza kurroa, Origanum vulgare,* and *Ajuga parviflora* were subjected to antibacterial activity against the selected bacteria (E. *coli* and *S. aureus*), and fungi (*A. niger* and *P. chrysogenum*) by well diffusion assay. Selection of the plants was done randomly after plant survey from HRDI based on reported medicinal properties and those that are being cultivated at larger scale in Uttarakhand. 25 mg/L to 100 mg/L concentrations of leaf extracts were used in all antimicrobial assay. Results were also compared with standard antibiotic drug streptomycin (bacterial) and ketoconazole (fungi) on similar concentrations. Inhibition zone (IZ) and activity index (AI) were calculated for each concentration. Results revealed that all the selected plants showed potential antimicrobial effect against the selected bacteria and fungi. The highest activity was shown against *Aspergillus niger.* Against the fungus, *Artemisia annua* and *Artemisia vulgare* showed activity higher than the standard antifungal drug (AI-1.15 and 1.10 respectively) while at the similar concentration, *Origanum vulgare* and *Ajuga parviflora* showed activity similar to the antibiotic (AI-1.00 for both). The study reveals that the antimicrobial potential of the herbal plants will be used for the formulation new antimicrobial drugs.

Keywords: Valley of flowers, antimicrobial drugs, antimicrobial assay, medicinal herbs

Introduction

In the recent past, WHO reported that the infections with drug-resistant bacteria were responsible for the higher threat of death rate at global level[1]. Increase in consumption of antimicrobial agent, inappropriate use of antibiotics and also the continuous migration of people leads to increasing of multidrug-resistant microbial strains[2]. A significant public health issue is the worldwide incidence of microbial infections and illnesses[3]. Due to their similar safety and efficacy, plants are being studied for their potential as antimicrobial substances against resistant strains as a result of the recent growth of antibiotic resistance and associated toxicity challenges, which constrain the effective application of antimicrobial compounds [4,5]. In nations with limited resources, between 60

and 90 % of the populace takes medicine obtained from plants. Crude plant extracts have historically been utilised as herbal medicine to treat viral disorders in humans[6].

Natural goods are the source of more than half of all contemporary clinical medications, and they are crucial to pharmaceutical companies' in drug development initiatives[7]. According to Jayapriya and Gricilda[8], medicinal plants are thought to be a significant source of novel compounds for drug development as well as a possible source of novel compounds with therapeutic applications. In order to defend themselves against bacteria and fungi, these plants produce a variety of chemical substances that function on the human body similarly to allopathic medications[9,10,11].

Numerous phytochemicals isolated from plants, such as flavonoids, terpenoids, alkaloids, and tannins, have been shown to have antibacterial and antifungal potential. These formulations mediate significant host responses, even though the mechanism of action and efficacy of these herbal extracts in most situations remain required to be verified scientifically[12,13].

In the present study, ethanolic extracts of leaves of 5 herbal medicinal plants (collected from Uttarakhand, India) were evaluated against some bacteria and fungi.

Materials and methods

Collection and extraction of plant materials

Leaves of *Artemisia annua, Artemisia vulgare, Picrorhiza kurroa, Origanum vulgare* and *Ajuga parviflora* were collected and authenticated from Herbal Research and Development Institute (HRDI), Uttarakhand. Selection of the plants was done randomly after collection of some plants from HRDI based on their medicinal properties as those are being cultivated at larger scale in Uttarakhand. Those were washed with distilled water and then air dried at room temperature. After complete drying plant materials were grinded to make fine powder and stored for further use.

1 gm of the grinded plant material was dissolved in 10 ml of ethanol and kept in sonicator for 10 minutes at 40°C temperature[14]. After that, extract were filtered (Whatmann Filter paper No. 1), and solvent was evaporated to obtain dry extract. Extractive values were calculated as mg/g.dw for each.

Evaluation of antimicrobial activity

Standard microbial technique - the agar well diffusion method was used for *in-vitro* antimicrobial assay[15-19]. For antibacterial activity, nutrient agar was used while for antifungal activity, potato dextrose agar was used. The different samples were diluted by using dimethyl sulphoxide (DMSO) and 4 different concentrations (25 mg/L, 50 mg/L, and 75 mg/mL and 100 mg/mL) of all compounds were prepared. Disinfected petri dishes holding the nutrient agar (NA) medium were used for the inoculation of test microorganisms, this inoculum spread all over the dish using spreader and kept standing for 30 min. Wells of 6 mm diameter were prepared in the seeded agar plates. In a different petri plate, standard drug was also loaded at similar concentrations. All different concentrations of all the samples and standard drug (30 µl in each well) poured into the preorganized wells of seeded plates. The plates were kept for incubation at 37°C for 24 hrs. The antibacterial spectrum of the test sample was determined via inhibition zone (IZ) around each prepared well. The comparison of diameters of inhibition zone developed by the test sample and by the commercial control antibiotics (streptomycin and ketoconazole for antibacterial and antifungal respectively) was done.

Activity Index was calculated from comparison of activity of samples with the standard antibiotic drug. The activity index was calculated by the following formula:

Activity Index (AI) = Inhibition zone of sample/Inhibition zone of standard

Results

In each plant extractive values in ethanol was measured as mg/g in dry weight of plant. Results are shown in table 1. It was observed that among the selected plants, *Artemisia vulgare* showed maximum value (0.032 mg/g.dw) followed by *Artemisia annua* (0.018 mg/g.dw), *Ajuga parviflora* (0.017 mg/g.dw), *Picrorhiza kurroa* (0.011 mg/g.dw) and *Origanum vulgare* (0.009 mg/g.dw).

Against *E. coli*, all plants showed good activity except *Ajuga parviflira* which did not show any activity at the lowest concentration (25 mg/L). The highest activity was shown by *Origanum vulgare* (AI- 0.45 to 0.53) followed by *Artemisia vulgare* (AI- 0.33 to 0.53), *Artemisia annua* (AI- 0.29 to 0.37) and *Picrorhiza kurroa* (AI- 0.29 to 0.34) (Table 2).

Against *S. aureus*, all the plant parts showed inhibitory activity at the selected concentrations. The maximum activity was shown by *Picrorhiza kurroa* (AI- 0.35 to 0.44) followed by *Artemisia annua* (AI-0.25 to 0.39), *Origanum vulgare* (AI-0.22 to 0.42) and *Artemisia vulgare* (AI-0.22- 0.39) (Table 3).

Against *A. niger*, all the selected plants showed potential antimicrobial activity. At their highest concentrations, both *Artemisia annua* and *Artemisia vulgare* showed activity higher than the standard antifungal drug (AI-1.15 and 1.10 respectively) while at the similar concentration, *Origanum vulgare* and *Ajuga parviflora* showed activity similar to the antibiotic (AI-1.00 for both). *Picrorhiza kurroa* showed activity index from 0.53 to 0.94 (Table 4).

Against *Penicillum chrysogenum*, all plant extracts showed efficient inhibitory potential. All extracts showed almost similar antimicrobial potential. The activity index range for *Artemisia annua, Artemisia vulgare, Picrorhiza kurroa, Origanum vulgare* and *Ajuga parviflora* were 0.35 to0.50, 0.35 to 0.41, 0.35 to 0.50, 0.40 to 0.50 and 0.35 to 0.45 respectively (Table 5).

Table1: Extractive values of leaves of the selected plants in ethanol

Plant name	*Artemisia annua*	*Artemisia vulgare*	*Picrorhiza kurroa*	*Origanum vulgare*	*Ajuga parviflora*
Extractive value (mg/g dry weight)	18	32	11	9	17

Table 2: Antibacterial activity of the selected plant extracts against *E. coli.*

	Activity at different concentrations (mg/L)							
	25		**50**		**75**		**100**	
Name of plant	**IZ (mm)**	**AI**	**IZ (mm)**	**AI**	**IZ (mm)**	**AI**	**IZ (mm)**	**AI**
Artemisia annua	7	0.29	8	0.26	9	0.29	12	0.37
Artemisia vulgare	8	0.33	10	0.33	15	0.48	17	0.53
Picrorhiza kurroa	7	0.29	8	0.26	10	0.32	11	0.34
Origanum vulgare	11	0.45	13	0.43	16	0.51	17	0.53
Ajuga parviflora	NA	NA	7	0.23	9	0.29	10	0.31
Standard	24		30		31		32	

Table 3: Antibacterial activity of the selected plant extracts against *S. aureus.*

	Activity at different concentrations (mg/L)							
	25		**50**		**75**		**100**	
Name of plant	**IZ**	**AI**	**IZ**	**AI**	**IZ**	**AI**	**IZ**	**AI**
Artemisia annua	8	0.25	12	0.34	14	0.38	15	0.39
Artemisia vulgare	7	0.22	10	0.28	13	0.36	15	0.39

Picrorhiza kurroa	11	0.35	13	0.37	15	0.41	17	0.44
Origanum vulgare	7	0.22	8	0.22	15	0.41	16	0.42
Ajuga parviflora	NA	NA	NA	NA	8	0.22	10	0.26
Standard	31		35		36		38	

Table 4: Antifungal activity of the selected plant extracts against *A. niger.*

	Activity at different concentrations (mg/L)							
	25		50		75		100	
Name of plant	IZ	AI	IZ	AI	IZ	AI	IZ	AI
Artemisia annua	7	0.46	10	0.62	21	1.23	22	1.15
Artemisia vulgare	9	0.6	11	0.68	15	0.88	21	1.10
Picrorhiza kurroa	8	0.53	10	0.62	15	0.88	18	0.94
Origanum vulgare	8	0.53	9	0.56	11	0.64	19	1
Ajuga parviflora	9	0.6	11	0.68	16	0.94	19	1
Standard	15		16		17		19	

Table 5: Antifungal activity of the selected plant extracts against *P. chrysogenum.*

	Activity at different concentrations (mg/L)							
	25		50		75		100	
Name of plant	IZ	AI	IZ	AI	IZ	AI	IZ	AI
Artemisia annua	7	0.35	8	0.38	10	0.43	12	0.5
Artemisia vulgare	7	0.35	8	0.38	9	0.39	10	0.41
Picrorhiza kurroa	7	0.35	9	0.42	11	0.47	12	0.5
Origanum vulgare	8	0.4	9	0.42	11	0.47	12	0.5
Ajuga parviflora	7	0.35	8	0.38	10	0.43	11	0.45
Standard	20		21		23		24	

Discussion

The problem of microbial resistance is growing by time and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to face this problem and to introduce the antibacterial activity of medicinal plants[20–26].

In the present study, *Artemisia annua, Artemisia vulgare, Picrorhiza kurroa, Origanum vulgare* and *Ajuga parviflora* leaf extracts were evaluated for their inhibitory potential against bacteria (*E. coli* and *S. aureus*) and fungi (*Aspergillus niger* and *Penicillum chrysogenum*). The obtained inhibition zones were compared with standard antibiotic drugs. It was found that all plant extracts showed efficient antibacterial and antifungal activity. Even against *Aspergillus niger*, those plant extracts showed activity higher or approximately similar to antibiotic drug.

Due to having antimicrobial properties, plant extracts can serve as natural preservatives in different food products, and they are gaining a tremendous amount of attention due to the deleterious effects of the synthetic counterparts[27]. Antimicrobials from medicinal plant extracts can be applied alone or in combination with standard antibiotics to achieve bactericidal synergism to broaden the antimicrobial spectrum, prevent the emergence of drug-resistant mutants, and minimize toxicity[28]. Despite all the benefits associated with using antimicrobials from medicinal plant extracts, there are some challenges when using them in a practical setting, including the absence of standardization in treatments and difficulties in reproducing the consistent

composition of plant extracts. To overcome these limitations, studies on the pharmacology of medicinal plant-derived compounds must be studied to achieve the standardization of the therapeutic regimens and the characterization of bioactive compounds to establish quality control procedures[29].

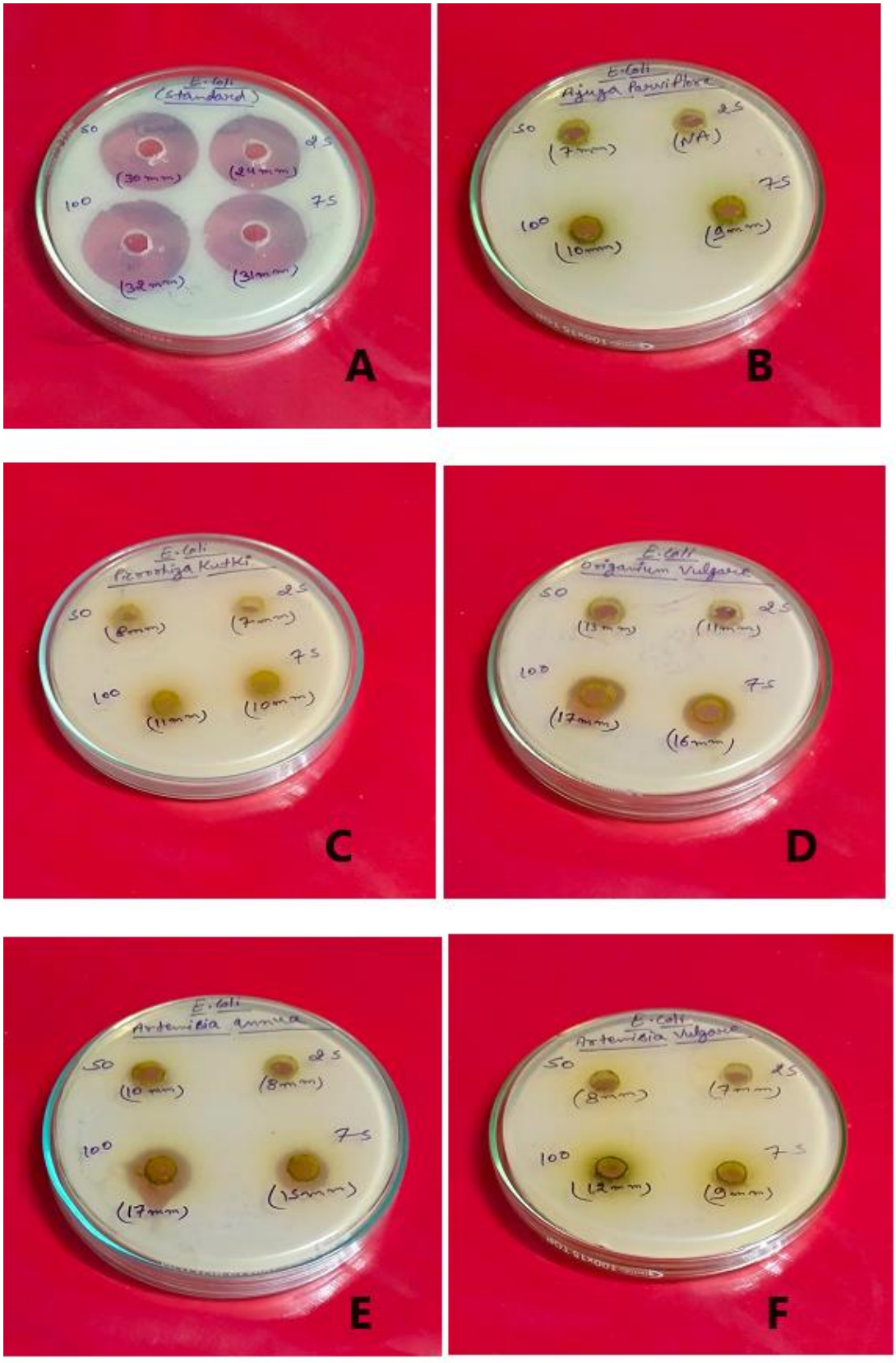

Figure 1: Antibacterial activity of the selected plant extracts against *E. coli.*

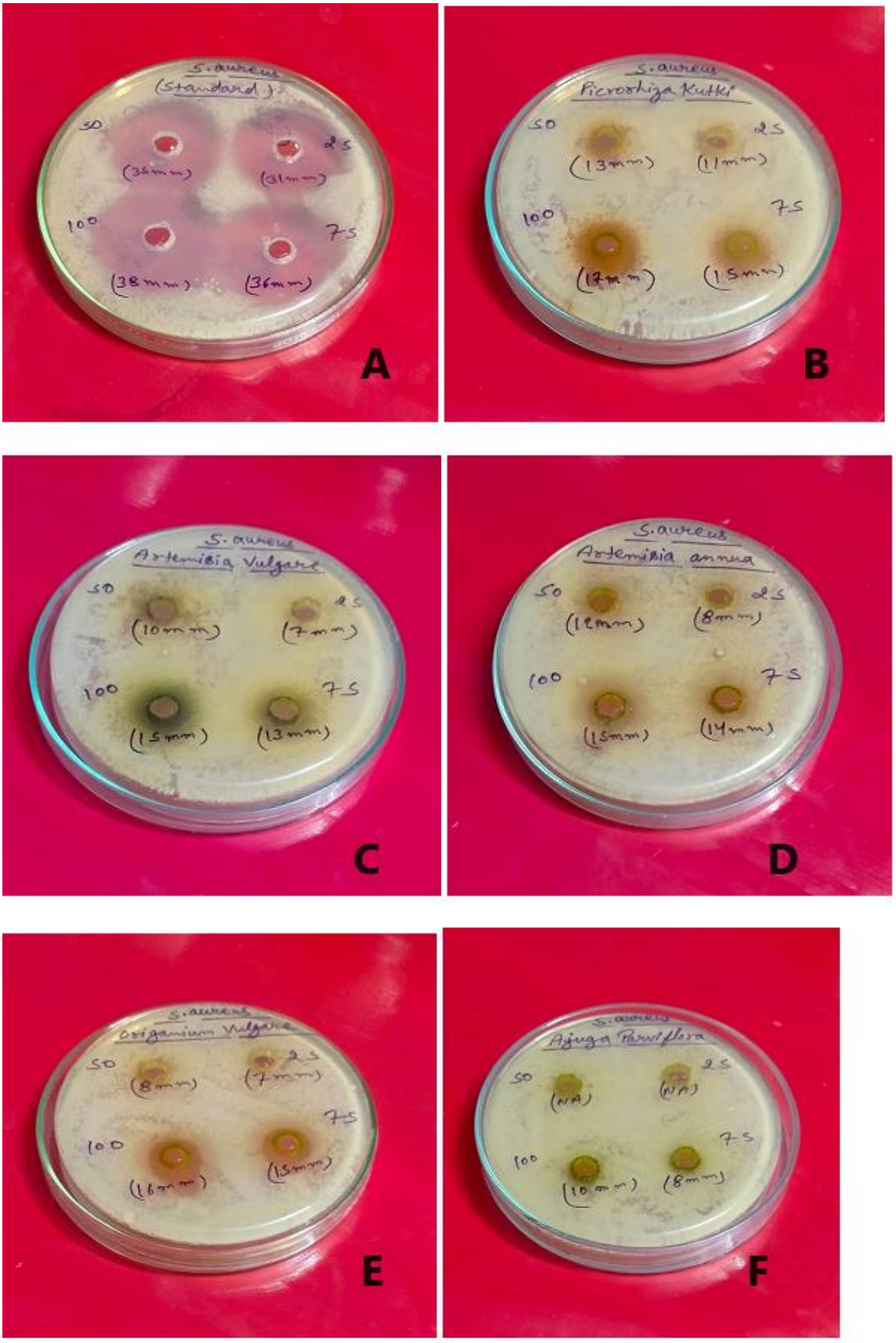

Figure 2: Antibacterial activity of the selected plant extracts against *S. aureus.*

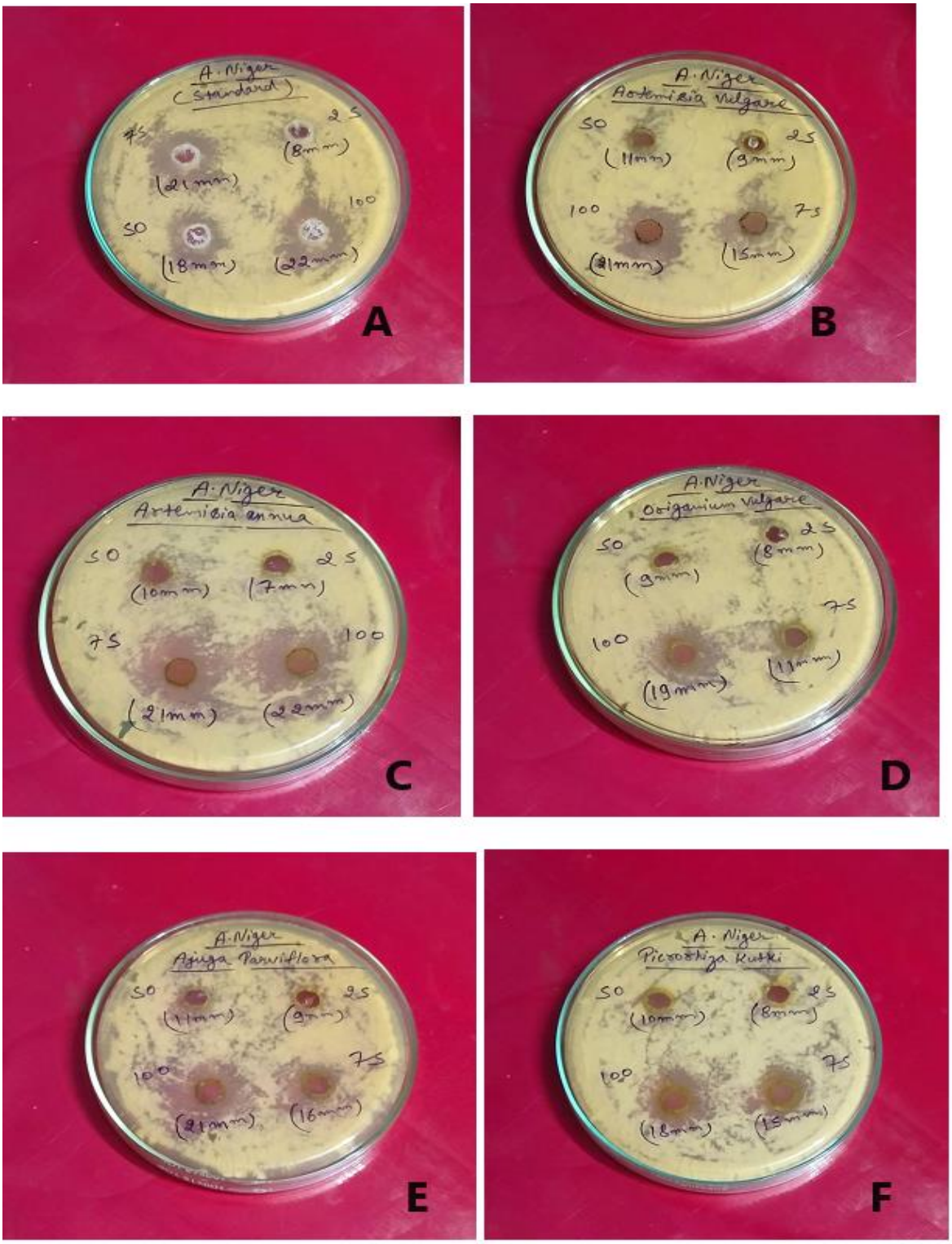

Figure 3: Antifungal activity of the selected plant extracts against *A. niger.*

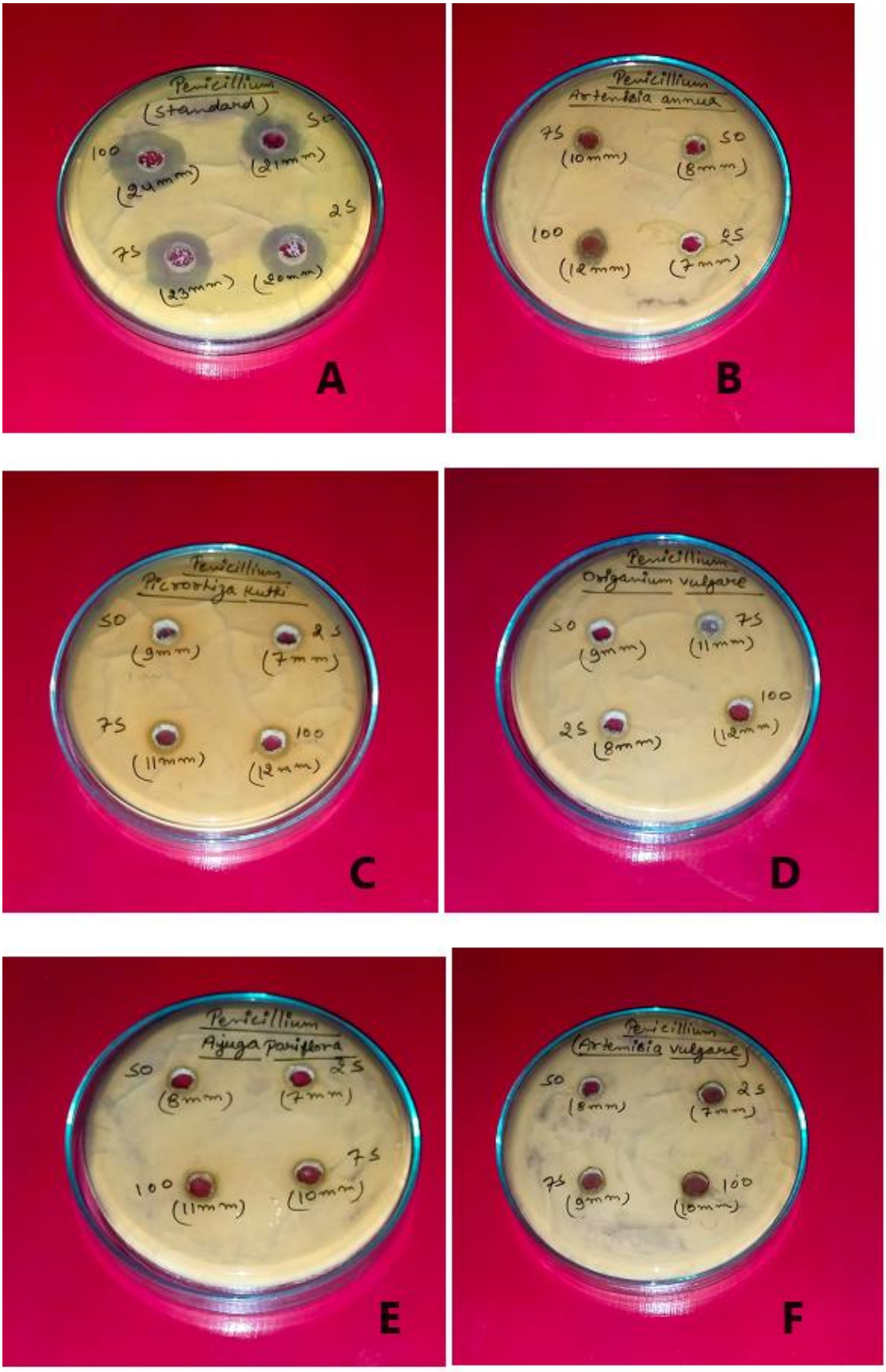

Figure 4: Antifungal activity of the selected plant extracts against *P. chrysogenum*

Conclusion

The antimicrobial activities may be due to the strong occurrence of active compounds, i.e., saponins, tannin, alkaloids, steroids, phenols, and flavonoids. It has been concluded that extracts are suitable candidates for the development of novel antimicrobial compounds. The results showed that different plants have considerable antibacterial as well as antifungal action against the selected microorganisms. Further phytochemical analysis of these plant parts will be helpful for the elucidation of lead molecules in it may be employed as an eco-friendly, biodegradable alternative to prevent and treat bacterial cum fungal infections.

References

1. Baptista, P. V., McCusker, M. P., Carvalho, A., Ferreira, D. A., Mohan, N. M., Martins, M., & Fernandes, A. R. (2018). Nano-strategies to fight multidrug resistant bacteria—"A Battle of the Titans." Frontiers in Microbiology, 9, 1441.
2. Nadeem, S. F., Gohar, U. F., Tahir, S. F., Mukhtar, H., Pornpukdeewattana, S., Nukthamna, P., Moula Ali, A. M., Bavisetty, S. C. B., & Massa, S. (2020). Antimicrobial resistance: More than 70 years of war between humans and bacteria. Critical Reviews in Microbiology, 46(5), 578-599.
3. Williamson, D.A., Heffernan, H., Sidjabat, H., Roberts, S.A., Paterson, D.L., Smith, M., (2008). Impact of antibiotic resistance in gram-negative bacilli on empirical and definitive antibiotic therapy. Clin. Infect. Dis. 1: 14 – 20.
4. Bameri, Z., Amini-Boroujeni, N., Saeidi, S., Bazi, S., (2013). Antibacterial activity of *Cassia angustifolia* extract against some human pathogenic bacteria. J. Nov. Appl. Sci. 2(11): 584- 6.
5. Alka, R Sanjeevi, J Anuradha, Sharma Madhu, (2024). Biofilm Formation of *Acinetobacter baumannii* in Clinical Samples from Tertiary Care Hospital. African Journal of Biological Sciences, 6(9): 4218-4235.
6. Bhargava, S., Dhabhai, K., Batra, A., Sharma, A., Malhotra, B., (2012). *Zingiber Officinale*: Chemical and phytochemical screening and evaluation of its antimicrobial activities. J. Chem. Pharm. Res. 4(1): 360 – 4.
7. Sheeba, J.B., Selva-Mohan, T., (2012). Antimicrobial activity of *Adhatoda vasica* against clinical pathogens. Asian J Plant Sci Res. 2(2):83-88.
8. Jayapriya, G., Gricilda-Shoba, F., (2015). Phytochemical analysis, antimicrobial efficacy and determination of bioactive components from leaves of *Justicia adhatoda.* Asian J Plant Sci Res. 5(2):43-51.
9. Nithyatharani, R., Kavitha, U.S., (2018). Phytochemical analysis of the leaves of *Adhatoda vasica*. Int J Creat Res Thoughts. ISSN 2320-2882.
10. Chaudhary, E., Chauhan R, Chauhan, N., (2017). Assessment of bactericidal and Phytochemical Properties of *Adhatoda vasica* Various Extracts against gram positive and Gram-negative Bacteria. Int J Curr Microbiol Appl Sci. 6(7):2660- 2669.
11. Gohel, A., Upadhye, V., Tarun, K., Upadhyay, Esha-Rami, Rakeshkumar, P., Suraj, J., Rajesh, D., Vijay, K., (2021). Study on Phytochemical Screening and Antimicrobial Activity of *Adhatoda Vasica*. Canadian Journal of Medicine, 3, 105-11.
12. Rahmoun, N.M., Ziane, H., Boucherit-Otmani, Z., (2014). Antibacterial and antifungal screening of four medicinal plants. J. Coast Life Med. 2(12): 975 – 9.
13. Perez, C., Anesini, C., (1994). Antibacterial activity of alimentary plants against Staphylococcus aureus growth. Am J Chin Med. 22(20:169-74.

14. Ranjha, M. M. A., Irfan, S., Lorenzo, J. M., Shafique, B., Kanwal, R., Pateiro, M., ... & Aadil, R. M. (2021). Sonication, a potential technique for extraction of phytoconstituents: A systematic review. Processes, 9(8): 1406.
15. R Sanjeevi, Muhammad Bello Haruna, Sandeep Tripathi, Bipin Kumar Singh, J Anuradha (2017). Impacts of Global Carbon Foot Print on Marine Environment. International Journal of Engineering Research and Technology, Vol. (5), 5 57-60.
16. Osman, K.M., Marouf, S.H., AlAtfeehy, N., (2013). Antimicrobial resistance and virulence-associated genes of *Salmonella enterica* subsp. Enterica serotypes Muenster, Florian, Omuna, and Noya strains isolated from clinically diarrheic humans in Egypt. Microbial Drug Resist. (5):19370-19377.
17. Mishra, S., Anuradha, J., Tripathi, S. and Kumar, S., 2016. In vitro antioxidant and antimicrobial efficacy of Triphala constituents: Emblica officinalis, Terminalia belerica and Terminalia chebula. Journal of Pharmacognosy and Phytochemistry, 5(6), pp.273-277.
18. Sadhana Rai, R Sanjeevi, J Anuradha, (2024). Assessment of Antibacterial and Antifungal Efficacy of Endophytic Fungi Isolated from *Psoralea corylifolia* Linn. Journal of Advanced Zoology. 45(2): 571-579.
19. Rai S, Anuradha J, Sanjeevi R (2023). Investigation On the Larvicidal Potential of Endophytic Fungi Isolates from Psoralea corylifolia and Leptadenia reticulata Against Aedes Aegypti. Journal of Advanced Zoology. 44, Pg 255.
20. Sadhana Rai, J. Anuradha, R. Sanjeevi, Madhvi Sharma (2023). Phytochemicals, Pharmacological aspects of Medicinal herbs: Leptadenia reticulata (Retz.) Wight & Arn. and Psoralea corylifolia L. 44(11), Pg. 939-960.
21. H Ranjan, R Sanjeevi, SP Vardhini, S Tripathi, J Anuradha, 2023. Biogenic Production of Silver Nanoparticles Utilizing an Arid Weed (Saccharum munja Roxb.) and Evaluation of its Antioxidant and Antimicrobial Activities, European Chemical Bulletin 12 (6), 911-921.
22. H Ranjan, R Sanjeevi, SP Vardhini, S Tripathi, J Anuradha, 2023. Eco-friendly Synthesis of Silver Nanoparticles using Capparis decidua (Forsk.) Edgew Stem Extract: An Investigation for Potential Antimicrobial Agent, European Chemical Bulletin 12 (12), 142-150.
23. Alka, R Sanjeevi, J Anuradha, M Sharma, 2023. Insights of Antimicrobial Resistant Acinetobacter baumannii and its Role in Biofilm Formation Causing Pathogenicity, European Journal of Molecular & Clinical Medicine 10 (1), 1726-1743.
24. Ramakrishnan S, Jayaraman A. Pesticide Contaminated Drinking Water and Health Effects on Pregnant Women and Children. In Research Anthology on Advancements in Women's Health and Reproductive Rights 2022 (pp. 682-696). IGI Global.
25. Abbasi T, Anuradha J, Abbasi SA. Nanotechnology and its potential in revolutionizing the pollution control scenario. Journal of the IPHE. 2009 Dec; 10: 5-12.
26. Freeda, R.R., and Padua, J.C., (2022). *In Vitro* Evaluation of Antimicrobial Activity of *Justicia Adhatoda* Aqueous Leaf Extract Cross Res. Vol.13 No.2.