



ORIGINAL ARTICLE

Antimicrobial Activities of Leaf Extract of some Hemi parasitic Taxa against some Common Human Pathogen: First time report in the World

Souradut Ray, Sayantan Tripathi, Amal Kumar Mondal* and Nagendra Kumar Verma

UGC-DRS-SAP Department of Botany & Forestry,

Plant Taxonomy, Biosystematics and Molecular Taxonomy Laboratory

Vidyasagar University,

Midnapore- 721102, West Bengal, India

E mails: amalcaebotvu@gmail.com*, souradutr@gmail.com tripathi.sayantan40@gmail.com;

ABSTRACT

Dendrothoe falcata, *Macrosolon cochichinensis*, *Viscum articulatum*, *Loranthus parasiticus* and *Cassytha filiformis* are hemi parasites found in the forests of South West Bengal, showed antimicrobial activities against four bacterial strains (*Bacillus subtilis*, *Klebsella pneumoniae*, *Vibrio cholerae* and *Escherichia coli*). The methanolic solution of the leaf extracts of *Dendrothoe falcata*, *Viscum articulatum*, *Cassytha filiformis* showed significant antimicrobial activity whereas acetonic solution of the extract of *Macrosolon cochichinensis*, *Loranthus parasiticus* showed good result against the strains. *Macrosolon cochichinensis* showed maximum antimicrobial activity with the inhibition zone ranging from 2- 16 mm followed by *Viscum articulatum* with inhibition zone 2- 14 mm and *Loranthus parasiticus* with inhibition zone ranging from 1-11 mm. *Dendrothoe falcata* and *Cassytha filiformis* on the other hand showed least activity with inhibition zone ranging from 2-8 mm and 1-7 mm respectively.

Key words: Hemiparasitic taxa, Antimicrobial activities, Inhibition zone, South West Bengal.

Received 20.04.2014

Revised 25.04.2014

Accepted 02.05. 2014

INTRODUCTION

Various plants and their parts are used for medicinal purpose from ancient age. Recently it has been reported that the African gorillas also use various plants and their parts to cure themselves [1]. The medicinal plants have been used by Hakims and in folklore medicines, as 80% of the population lives in rural areas that mostly depend on Unani system of medicines [2]. The potential of higher plants as source of new drug is still largely unexplored. Among the estimated 250,000-500,000 plant species, only a small percentage has been investigated phytochemically and the fraction submitted to biological or pharmacological screening is even smaller. Thus, any phytochemical investigation of a given plant will reveal only a very narrow spectrum of its constituents. Historically pharmacological screening of compounds of natural or synthetic origin has been the source of innumerable therapeutic agents. Random screening as a tool in discovering new biologically active molecules has been most productive in the area of antibiotics. Even now, contrary to common belief, drugs from higher plants continue to occupy important niche in modern medicine. On global basis, at least 130 drugs, all single chemical entities extracted from higher plants or modified synthetically are used.

Plants remain the most common source of antimicrobial agents. Their use in traditional health remedies is very popular all over the world. Tribal people frequently use plants to treat common infectious diseases, and some of these traditional medicines are still part of the habitual treatment of various diseases. The plant extracts have been developed and prescribed for use as antimicrobial substances [3]. Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper rate than the modern medicines [4]. Many workers have characterized different types of medicinal plants for their antioxidant and antimicrobial properties [5,6 and 7]. Although hundreds of

plant species have been tested for antimicrobial properties but vast majority of them have not been adequately evaluated [8].

Antimicrobial activities of many plants have been reported [9-12]. Even the antimicrobial effects of some hemi parasitic plant is known [13]. Hemi parasites are the type of parasitic plants, which carry on some photosynthesis but obtain a portion of food, water or minerals from the host plant. The tribal people of south West Bengal use some hemi parasites to cure their health problems. The camouflaging nature of these species make them further strenuous to locate in the forest. They grow on the stem of their host but they look like a branch of the tree (14).

Considering the vast potentiality of plants as sources for antimicrobial drugs with reference to antibacterial and antifungal agents, a systematic investigation was undertaken to screen the local hemi parasitic flora; *Dendrophoe falcata*, *Macrosolon cochichinensis*, *Viscum articulatum* *Loranthus parasiticus*, *Cassytha filiformis* for their antibacterial and antifungal activities.

Study area : A preliminary survey was made in different rural areas of the lateritic zone of South West Bengal from March 2012 to April 2012. The places under the zone of survey included Gar Panchkot (23°37'24" N latitude and 86°45'53" E longitude) and Ajodhya Hills (23°13'54"N latitude and 86°5'36" E longitudes). Plants or plant parts were collected from the different areas. Field information regarding their habit, habitat, dominance, local uses and their ethno-medicinal uses are based on personal observation and detailed discussions made with the tribal people inhabiting these areas, specially the aged people and the ojhas (quack doctors) during the period of regular field visits.

MATERIALS AND METHODS

Collection and preparation of plant material for extraction

Plant parts were washed with 70% alcohol and then rinsed with sterilized distilled water and air dried. Clean dry plant samples were stored in cotton bags. The materials were homogenized to fine powder with the help of a mixer grinder.

Preparation of acetone extracts

10 g of powdered material of each sample was soaked in 30 ml of acetone and kept at 37°C for 24 h on a rotary shaker. After 24 h the previous portion of added acetone was evaporated and the same volume of acetone was again added and placed on a rotary shaker for another 24 h at 37°C. It was then filtered with the help of a Whatman No. 1 filter paper. The filtrate was centrifuged at 2000 rpm for 10 minutes. The supernatant was then collected and allowed to evaporate until it was completely dry. The extracts were kept in sterile air tight bottles at 4°C until further use. Before use, 30 mg of dry extract was re-suspended in 1 ml of acetone so that the final concentration of the extract was 30 mg/ml [15].

Preparation of methanolic extracts

10 g of powdered material of each sample was soaked in 30 ml of 70% methanol and kept at 37°C for 24 h on a rotary shaker. After 24 h the previous portion of added methanol was evaporated and the same volume of methanol was again added and placed on a rotary shaker for another 24 h at 37°C. It was then filtered with the help of a Whatman No. 1 filter paper. The filtrate was centrifuged at 2000 rpm for 10 minutes. The supernatant was then collected and allowed to evaporate until it was completely dry. The extracts were kept in sterile air tight bottles at 4°C until further use. Before use 30 mg of dry extract was re-suspended in 1 ml of 70% methanol so that the final concentration of the extract was 30 mg/ml [16].

Preparation of aqueous extracts

2 g of powdered material of each sample was soaked in 20 ml of distilled water and kept at 37°C for 24 h on a rotary shaker. It was then filtered with the help of a Whatman No. 1 filter paper. The filtrate was centrifuged at 2000 rpm for 10 min. The supernatant was then collected and allowed to evaporate until it was completely dry. The extracts were kept in sterile air tight bottles at 4°C until further use (17).

Bacterial strains

Pure cultures of four bacterial strains *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae* and *Vibrio cholerae* were used for the study.

Agar well diffusion

Antimicrobial activity was determined by the agar-well diffusion method. Mueller Hinton Agar was used as media. To standardize the inoculums density for sensitivity test, a Barium Sulphate (BaSO₄) turbidity standard, equivalent to 0.5 Mac Farland standard was used and was cultured on agar medium. Thereafter 6 mm diameter wells were punched in the agar plates. Aqueous and methanolic extracts (100 µl) of the different plant extracts were added to the wells. The plates were then incubated at 37°C for 24 h. After incubation the antimicrobial activity was evaluated by measuring the inhibition zone diameter observed. Each test was performed twice and the average of the results was taken [18].

RESULTS AND DISCUSSION

The result of the screening of plant extracts for antimicrobial activity is summarized in the Table No. 1. *Dendrophoe falcata* *Macrosolon cochichinensis* *Viscum articulatum* *Loranthus parasiticus* and *Cassytha filiformis* all exhibited different degrees of antimicrobial activities against different strains of bacteria. Aqueous extract of *Dendrophoe falcata* showed inhibition zone of 2mm against *Vibrio cholerae*, and *Klebsiella pneumoniae* and 3 mm against *Escherichia coli* after 24 h incubation but did not show any response against *Bacillus subtilis*. The result remained unchanged after 48 hrs incubation. Methanolic extract of *Dendrophoe falcata* showed inhibition zone of 4 mm against *Vibrio cholerae*, 6 mm against *Escherichia coli* and *Klebsiella pneumoniae* and 7 mm against *Bacillus subtilis* after 24 hrs incubation. The result remained unchanged after 48 hrs incubation except in case of *Escherichia coli* where inhibition zone extended to 8 mm. Acetone extract of *Dendrophoe falcata* showed inhibition zone of 5 mm each against *Vibrio cholerae*, *Escherichia coli*, and *Bacillus subtilis* and 4mm against *Klebsiella pneumoniae* after 24 hrs incubation. The result remained unchanged after 48 hrs incubation except in case of *Bacillus subtilis* where inhibition zone extended to 6 mm.

Aqueous extract of *Macrosolon cochichinensis* showed inhibition zone of 3 mm against *Vibrio cholerae* and *Escherichia coli* 4 mm against *Bacillus subtilis* and only 2 mm against *Klebsella pneumoniae* after 24 hrs incubation. The result remained unchanged after 48 hrs incubation. Methanolic extract of *Macrosolon cochichinensis* showed inhibition zone of 7 mm against *Vibrio cholerae* and *Escherichia coli*, 6 mm against *Bacillus subtilis* and 9 mm against *Klebsella pneumoniae* after 24 hrs incubation. The inhibition zone extended to 8 mm for both *E. coli* and *B. subtilis* whereas other remained unchanged. Acetone extract of *Macrosolon cochichinensis* showed inhibition zone of 6 mm against *Vibrio cholerae*, 5 mm against *Escherichia coli*, 16 mm against *Bacillus subtilis* and 3 mm against *Klebsiella pneumoniae* after 24 hrs incubation. The result remained unchanged after 48 hrs incubation except in case of *E. coli* where inhibition zone extend to 6 mm.

Aqueous extract of *Viscum articulatum* showed inhibition zone of 2 mm against *Vibrio cholerae*, 3 mm against *Escherichia coli*, *Bacillus subtilis* and *Klebsella pneumoniae* after 24 hrs incubation. The result remained unchanged after 48 hrs incubation. Methanolic extract of *Viscum articulatum* showed inhibition zone of 5 mm against *Vibrio cholerae*, 14 mm against *Escherichia coli*, 9 mm against *Bacillus subtilis* and 4 mm against *Klebsiella pneumoniae* after 24 hrs incubation. The inhibition zone further extended to 7mm in case of *V. cholerae* which remained unchanged in case of others.. Acetone extract of *Viscum articulatum* showed inhibition zone of 6 mm against *Vibrio cholerae*, 5 mm against *Escherichia coli*, 4 mm against *Bacillus subtilis* and 3 mm against *Klebsiella pneumoniae* after 24 hrs incubation. The result remained unchanged after 48 hrs incubation except in case of *E. coli* where the inhibition zone extended to 8 mm.

Aqueous extract of *Loranthus parasiticus* showed inhibition zone of 1 mm only against *Vibrio cholerae*. Methanolic extract of *Loranthus parasiticus* showed inhibition zone of 2 mm against *Vibrio cholerae*, *Escherichia coli*, and *Klebsiella pneumoniae* each and 5 mm against *Bacillus subtilis* after 24 hrs incubation. The inhibition zone extended to 7mm in case of *V. cholerae* and *B. subtilis* after 48 hrs which remained unchanged in case of others. Acetone extract of *Loranthus parasiticus* showed inhibition zones of 2 mm against *Vibrio cholerae*, *Escherichia coli* and *Klebsiella pneumoniae* each and 5 mm against *Bacillus subtilis* after 24 hrs incubation. The inhibition zone extended to 7 mm for both *V. cholerae* and *B. subtilis* after 48 hrs. but a secondary inhibition zone was also observed in case of *E. coli* and *K. pneumoniae* after 48 hrs which extended to 13 mm and 8 mm respectively.

Aqueous extract of *Cassytha filiformis* showed inhibition zone of 2 mm only against *K. pneumoniae* after 24 hrs but after 48 hrs it was observed 2 mm in case of *E. coli* and 5 mm against *K. pneumoniae*. Methanolic extract of *Cassytha filiformis* showed inhibition zone of 4 mm against *Vibrio cholerae*, 3 mm against *Escherichia coli* and *Bacillus subtilis* each and 7 mm against *Klebsella pneumoniae* after 24 hrs incubation. Secondary inhibition zone was observed in case of *V. cholerae* and *K. pneumoniae* after 48 hrs which extended to 10 mm and 9 mm further. Acetone extract of *Cassytha filiformis* showed inhibition zone of 1 mm against *Vibrio cholerae*, 4 mm against *E. coli* and 3 mm against *Klebsella pneumoniae* after 24 hrs incubation but no such zone was found in case of *B. subtilis*. The inhibition zone extended to 6 mm for both *V. cholerae* and *E. coli* and 4 mm for *K. pneumoniae*.

The result indicated that the plant extracts with different solvent groups have the potential to act against different bacterial strains. The possible reason might be the solubility of different components in different solvents. The aqueous extract of all the plant materials were observed less potent compared to methanolic and acetone extract. Methanolic extract of *Dendrophoe falcata*, *Viscum articulatum* and *Cassytha filiformis* showed the best result in comparison to other aqueous and acetonic extractions. But in case of *Macrosolon cochichinensis*, *Loranthus parasiticus* it was the acetone extraction which gave the best result in comparison to others.

The diameter of inhibition zone increase minutely from 24 hrs to 48 hrs and an interesting effect is found, after 48 hrs a secondary inhibition zone formed around the prominent inhibition zone. The secondary inhibition zones contain a small amount of bacterial colony which represents the strong effectiveness of leaf extract.

From this investigation, it is found that the bacteria *Vibrio cholerae* is highly inhibited by the methanolic solution of *Macrosolon cochichinensis*. *Bacillus subtilis* is greatly inhibited by acetonic solution of *Macrosolon cochichinensis*. *E.coli* is highly inhibited by methanolic solution of *Viscum articulatum*. *Klebsella pneumoniae* is greatly inhibited by methanolic solution of *Cassytha filiformis*.

Here by it can be stated that *Macrosolon cochichinensis* has the highest power to inhibit the bacterial colony as it shows its effectiveness against all the bacterial strains.

This investigation revealed that the bacterial inhibition can vary with the plant extract, the solvent used for extraction, and the organisms tested. From this point of view, methanolic solution is more effective than the other two.

All the plant extracts have the capacity to inhibit bacterial strains. The methanolic solution of *Dendrophthoe falcata* shows great inhibition against *B subtilis*. Acetonic extract of *Macrosolon cochichinensis* is very much active against *B subtilis*. Methanolic solution of *Viscum articulatum* is highly effective against *E.coli*. methanolic solution of *Loranthus parasiticus* shows great activity against *V. chloreae*. Methanolic solution of *Cassytha filiformis* is effective against *K.pneumoniae*.

Table 1. Antimicrobial effect of the aqueous extracts of the five plants.

Plant	Diameter of the inhibition zone (mm)							
	<i>Bacillus subtilis</i>		<i>Klebsiella pneumoniae</i>		<i>Vibrio cholerae</i>		<i>Escherichia coli</i>	
	after 24 hrs	after 48 hrs	after 24 hrs	after 48 hrs	after 24 hrs	after 48 hrs	after 24 hrs	after 48 hrs
<i>Dendrophthoe falcata</i>	0	0	2	2	2	2	3	3
<i>Macrosolon cochichinensis</i>	4	4	2	2	3	3	3	3
<i>Viscum articulatum</i>	3	3	3	3	2	2	3	3
<i>Loranthus parasiticus</i>	0	0	0	0	1	1	0	0
<i>Cassytha filiformis</i>	0	0	2	5	0	0	0	2

Table 2. Antimicrobial effect of the methanolic extracts of the five plants.

Plant	Diameter of the inhibition zone (mm)							
	<i>Bacillus subtilis</i>		<i>Klebsiella pneumoniae</i>		<i>Vibrio cholerae</i>		<i>Escherichia coli</i>	
	after 24 hrs	after 48 hrs	after 24 hrs	after 48 hrs	after 24 hrs	after 48 hrs	after 24 hrs	after 48 hrs
<i>Dendrophthoe falcata</i>	7	7	6	8	4	4	6	8
<i>Macrosolon cochichinensis</i>	6	8	9	9	7	7	7	8
<i>Viscum articulatum</i>	9	9	4	4	5	7	14	14
<i>Loranthus parasiticus</i>	5	7	2	2 (8)*	2	7	2	2 (13)*
<i>Cassytha filiformis</i>	3	3	7	7 (9)*	4	5 (10)*	3	3

Table 3. Antimicrobial effect of the acetonic extracts of the five plants.

Plant	Diameter of the inhibition zone (mm)							
	<i>Bacillus subtilis</i>		<i>Klebsiella pneumoniae</i>		<i>Vibrio cholerae</i>		<i>Escherichia coli</i>	
	after 24 hrs	after 48 hrs	after 24 hrs	after 48 hrs	after 24 hrs	after 48 hrs	after 24 hrs	after 48 hrs
<i>Dendrophthoe falcata</i>	5	6	4	4	5	5	5	5
<i>Macrosolon cochichinensis</i>	16	16	3	3	6	6	5	6
<i>Viscum articulatum</i>	4	4	3	3	6	6	5	8
<i>Loranthus parasiticus</i>	2	2 (9)*	3	11	6	6 (14)*	5	8
<i>Cassytha filiformis</i>	0	0	3	4	1	6	4	6

(--)*: Secondary inhibition zone

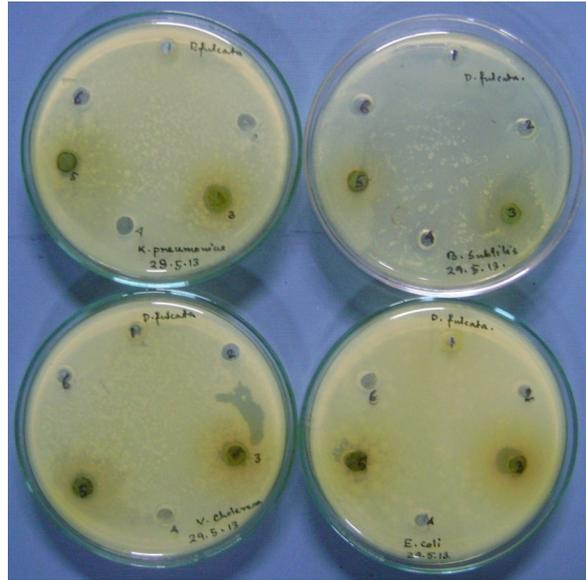


Fig- 1. Anti microbial activity of *Dendrothoe falcata* against all the four bacteria.

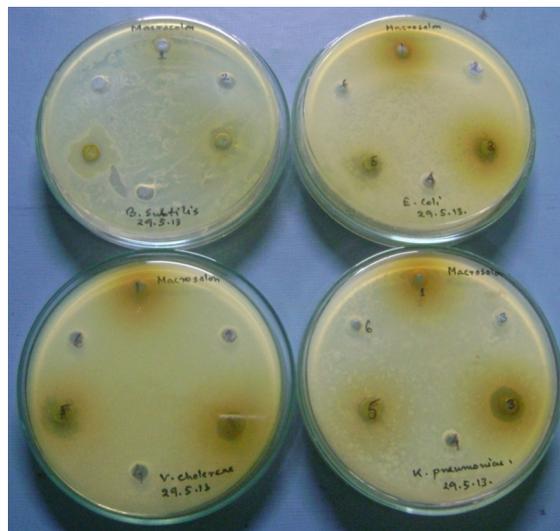


Fig- 2. Anti microbial activity of *Macrosolon cochichinensis* against all the four bacteria.

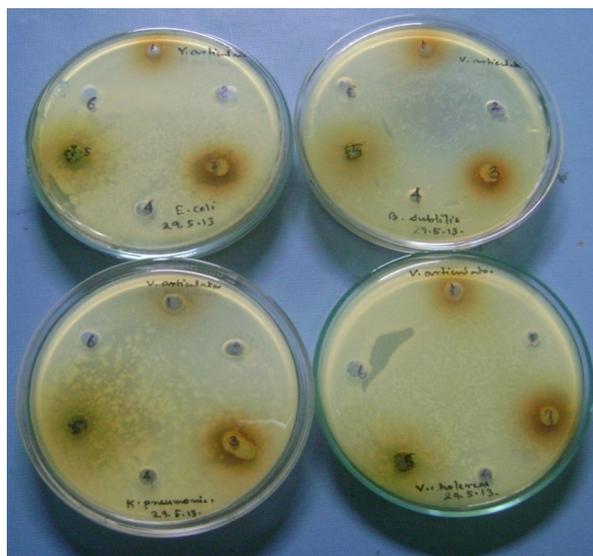


Fig- 3. Anti microbial activity of *Viscum articulatum* against all the four bacteria.

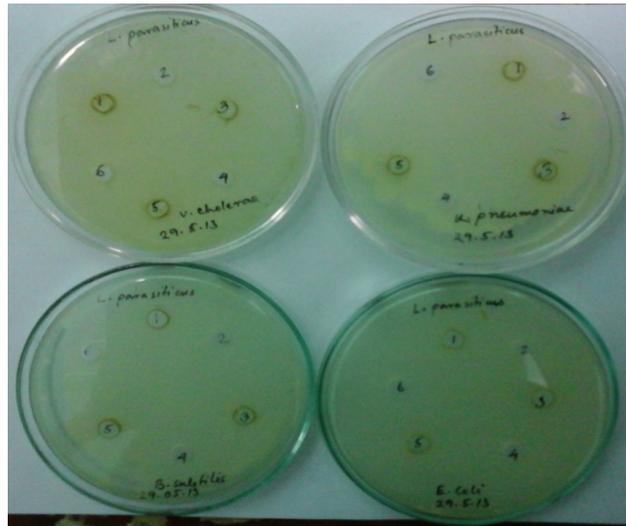


Fig- 4. Anti microbial activity of *Loranthus parasiticus* against all the four bacteria

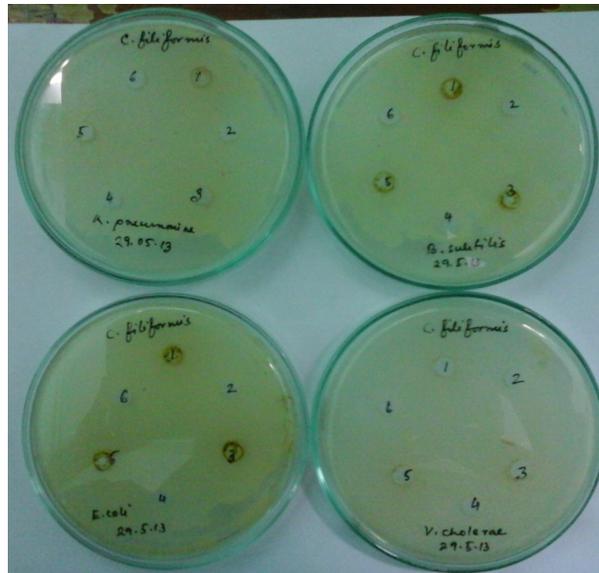


Fig- 5. Anti microbial activity of *Cassytha filiformis* against all the four bacteria

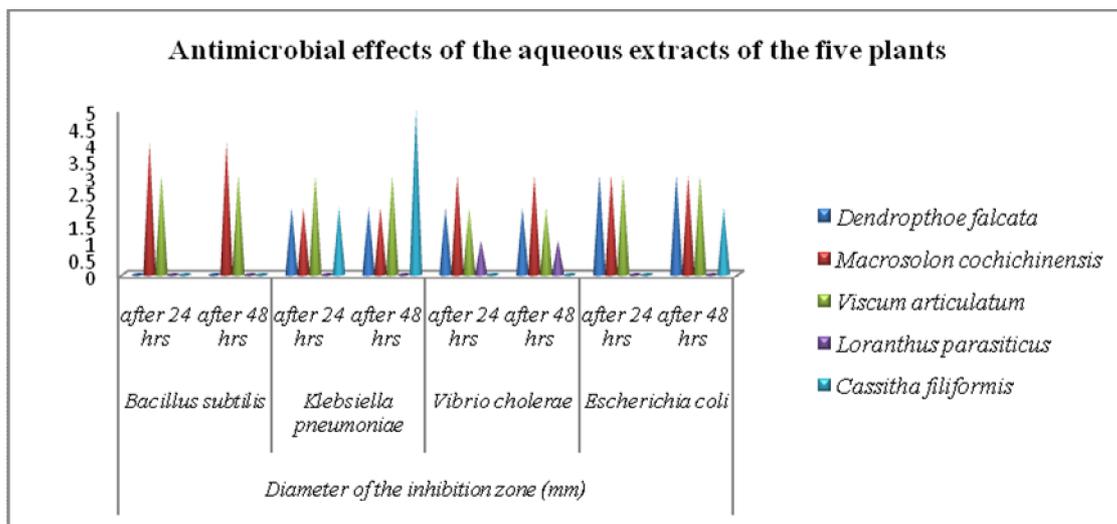


Fig- 6. Comparative analysis graph of Antimicrobial activity of the aqueous extracts of five hemi parasitic plants.

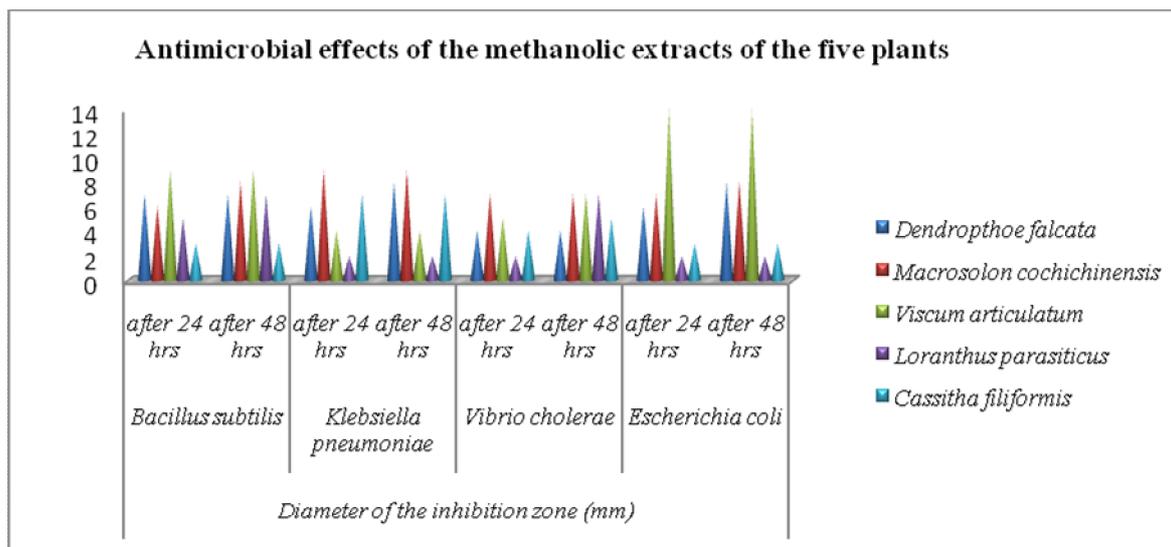


Fig- 7. Comparative analysis graph of Antimicrobial activity of the methnolic extracts of five hemi parasitic plants.

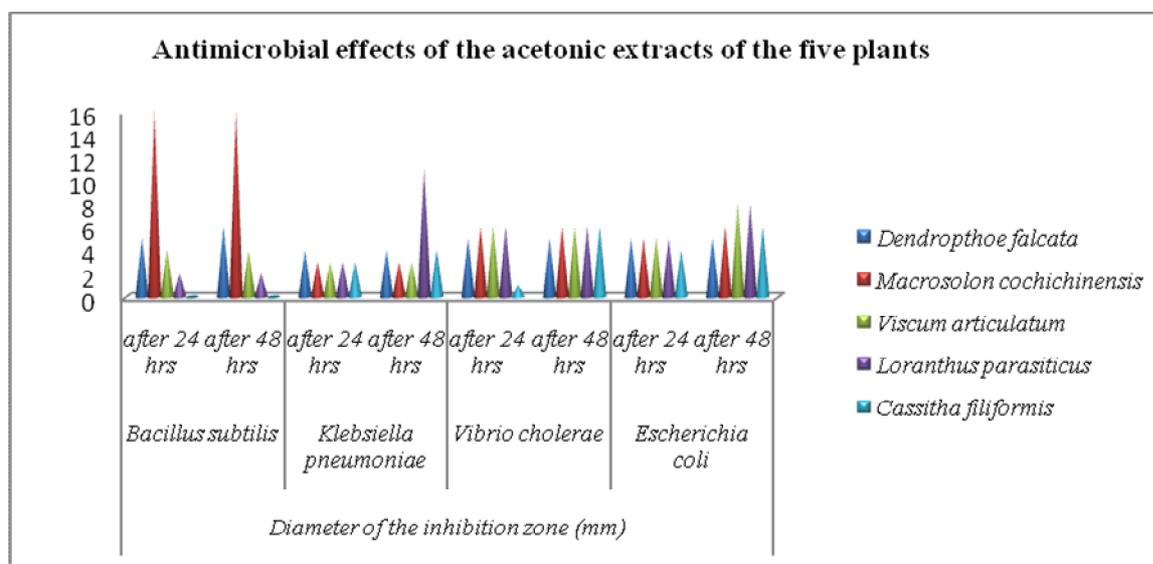


Fig- 8. Comparative analysis graph of Antimicrobial activity of the acetic extracts of five hemi parasitic plants.

CONCLUSION

The demonstration of broad spectrum of antibacterial activity by *Dendrothoe falcata*, *Macrosolon cochichinensis*, *Viscum articulatum*, *Loranthus parasiticus*, *Cassytha filiformis* may help to discover new chemical classes of antibiotic substances that could serve as selective agents for infectious disease chemotherapy and control. This investigation has opened up the possibility of the use of this plant in drug development for human consumption possibly for the treatment of gastrointestinal, respiratory tract and dysentery. The effect of these plants on more pathogenic organisms and toxicological investigations and further purification however, needs to be carried out.

ACKNOWLEDGEMENT

We are highly acknowledge to UGC for their financial assistance in the form of DRS-SAP (Phage-I) [2011-2016]. I also like to thank research scholars in the DRS-SAP Department of Botany & Forestry for their support in the form of collection of plant material due to which this paper has been completed successfully. We also acknowledge thankfully the suggestions given by Dr Sanjukta Mondal (Parui), Associate professor and Head, Department of Zoology (Biochemistry), Lady Brabourne college, Kolkata-700 017, West Bengal and Dr. Debdulal Banerjee of the Department of Botany and Forestry,

Vidyasagar University, Midnapore for providing the bacterial strains and assisting with the antimicrobial assay.

REFERENCES

1. Tripathi, S. and Mondal, A. K., Comparative (Quantitative and Qualitative) studies of stomata of selected six medicinally viable species of Cassia L. IJLPBR, 2012, **1(3)**, 104-113.
2. Soomro, R. R., Qureshi, A., Mahmood, M. T., Khan, M.A. and Makka, G. A., Ethobotanical uses of *Adhatoda vesica* in Chest Dwaseases. Hamdard Medicus. 1997, **38(1)**, 24-29.
3. Mann, A., Bansa, A. and Clifford, L. C., An antifungal property of crude plant extracts from *Anogewassus leiocarpus* and *Terminalia avicennioides*. Tanzania J. Health Res, 2008, **10(1)**, 34-38.
4. Del Campo, J., Amiot, M. J. and Nguyen, C., Antimicrobial effect of Rosemary extract. J. Food Protect, 2000, **63**, 1359-1368.
5. Mothana, R. A. A. and Lindequwast, U., Antimicrobial activity of some medicinal plants of the wasland Soqotra. J. of Ethnopharmacology, 2005, **96**, 177-181.
6. Bajpai, M., Pande, A., Tewari, S. K. and Prakash, D., Phenolic contents and antioxidant activity of some food and medicinal plants. International Journal of Food Sciences and Nutrition, 2005, **56(4)**, 287-291.
7. Wojdylo, A., Oszmianski, J. and Czemerys, R., Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chemwastry, 2007, **105**, 940-949.
8. Mahesh, B. and Satwash, S., Antimicrobial Activity of Some Important Medicinal Plant against Plant and Human Pathogens, World Journal of Agricultural Sciences, 2008, **4 (S)**, 839-843.
9. Reddy, P. S., Jamil, K. and Madhusudhan, P., Antibacterial activity of wasolates from *Piper longum* and *Taxus baccata*. Pharma. Biol, 2001, **39**, 236-238.
10. Ateb, D. A. and Erdourul, O. T., Antimicrobial activities of various medicinal and commercial plant extracts. Turk. J. Biol, 2003, **27**, 157-162.
11. Gunalan, G., Saraswathy, A. and Krwashnamurthy, V., Antimicrobial Activity of Medicinal Plant *Bauhinia variegata* Linn. IJBPS, 2011, **1(4)**, 400-408.
12. Doughari, J. H., Antimicrobial Activity of *Tamarindus indica* linn. Tropical Journal of Pharmaceutical Research, 2006, **5(2)**, 597-603.
13. Hussain, A. M., Khan, Q. M. and Hussain, N., Antimicrobial Screening of *Viscum Album* L. Extracts. IACSIT Press, Singapore. IPCBEE. 2011, **6(2)**, 203-208.
14. Tripathi, S. Ray, S., Das, P. K., Mondal, A. K. and Verma, N. K., antimicrobial activities of some rare aerial hemi parasitic taxa of South West Bengal, India. International Journal of Phytopharmacology, 2013, **4(2)**, 106-112
15. Ushimaru, P. I., Mariama, T. N., Luiz, C., Di Luciano, B. and Ary, F. J., Antibacterial activity of medicinal plant extract. Braz. J. Microbiol, 2007, **38**, 717-719.
16. Ushimaru, P. I., Mariama, T. N., Luiz, C., Di Luciano, B. and Ary, F. J., Antibacterial activity of medicinal plant extract. Braz. J. Microbiol, 2007, **38**, 717-719.
17. Das, P. K., Mondal, A. K. and Parui, S. M., Antimicrobial Activity of some Selected dye yielding plants in Eastern India. African Journal of Plant Science, 2011, **5(9)**, 510-520.
18. Ulusoylu, M., Öndersev, D.V., Soyogul, Ü., Gürkan, E. and Tuzlaci, E., The cytotoxic and the biological (antibacterial and antifungal) activities of *Centaurea iberica* and *Ferulago confuse*. J. Fac. Pharm. Gazi Univ, 2001, **18(2)**, 75-80.

CITATION OF THIS ARTICLE

Souradut R, Sayantan T, Amal K M and Nagendra K. V. Antimicrobial Activities of Leaf Extract of some Hemi parasitic Taxa against some Common Human Pathogen: First time report in the World. Bull. Env. Pharmacol. Life Sci., Vol 3 [6] May 2014: 21-28