Biochem. Cell. Arch. Vol. 21, No. 2, pp. 5127-5133, 2021	www.connectjournals.com/bca	*ISSN 0972-5075*
DocID: https://connectjournals.com/03896.2021.21.5127		*eISSN 0976-1772*

EVALUATION OF ANTIMICROBIAL ACTIVITY OF AQUEOUS AND ALCOHOLIC EXTRACT OF *BILWADI AGAD* AGAINST BACTERIAL STRAINS

Anupama Kumari[1], R. C. Tiwari[1]*, Ved Bhushan Sharma[1], Shashikant Tiwari[2] and Rakesh Bhutiani[3]

[1]Department of Agadtantra, Rishikul Campus, Uttarakhand Ayurvedic University, Haridwar, India.
[2]Department of Rognidan, Rishikul Campus, Uttarakhand Ayurvedic University, Haridwar, India.
[3]Department of Zoology and Environmental Sciences, Gurukul Kangri (Deemed to be University), Haridwar, India.
*e-mail : agadtantra12@gmail.com

(Received 10 May 2021, Revised 16 July 2021, Accepted 28 July 2021)

ABSTRACT : *Bilwadi agad* is one formulation mentioned in *Asthang Hridyam* chapter 36/84-85 and *Asthang Sangrah* chapter 42/87-88. This formulation has been found useful in the treatment of poisonous conditions of toxicity like *sarpa, luta, vrischik, mushak dansa, jwar, visuchika, ajeerana, gar visha* etc. The present research study was carried out with an objective to search a novel herbal preparation having antibacterial potentials. The aim of the study was to assess the antimicrobial activity by calculating the zone of inhibition on some pathogenic bacterial strains for this two different extract (aqueous and alcoholic) of bilwadi agad were prepared and analysed in lab with different concentration (5%,10%,15%) of extract on different bacterial strains. Antimicrobial study was seen on total 10 bacterial strains i.e *Escherichia coli* (MTCC: 40), *Staphylococcus aureus* (MTCC: 3160), *Pseudomonas aeruginosa* (MTCC: 424), *Salmonella typhimurium* (MTCC: 3231), *Enterobacter aerogenes* (MTCC: 2822), *Klebsiella pneumoniae* (MTCC: 39), *Salmonella paratyphi B* (ATCC: 10719), *Vibrio cholera* (MTCC: 3906), *Shigella dysenteriaes* (ATCC: 13313) and *Clostridium botulinum* (NCTC: 3815). Antimicrobial activity of Bilwadi agad was performed using "well diffusion method" against human pathogenic bacteria. By observing the samples, the activity index of bilwadi agad was found to be greater than 0.5 and this indicates a significant antimicrobial against defined microbes. Standard antibiotic was used as positive control in this study.

Key words : Antimicrobial, *Bilwadi agad*, bacterial strains, herbal, jwar, visha, zone of inhibition.

How to cite : Anupama Kumari, R. C. Tiwari, Ved Bhushan Sharma, Shashikant Tiwari and Rakesh Bhutiani (2021) Evaluation of antimicrobial activity of aqueous and alcoholic extract of *Bilwadi agad* against bacterial strains. *Biochem. Cell. Arch.* **21**, 5127-5133. DocID: https://connectjournals.com/03896.2021.21.5127

INTRODUCTION

In today's era infectious diseases are spreading day by day. Also in decades of dramatic progress the treatment and prevention, infectious diseases remain a major problem causing death and worsens the life of millions of people around the world. In spite of lot of improvement in field of microbiology, we are unable to answer this challenge. Drugs obtained from their natural sources, plays a substantial part in the prevention and treatment of diseases. In many developing countries, traditional medicines or drugs are one of the primary systems for general health care (Nayan and Shukla, 2011). In India, Ayurveda is one of the central systems of traditional medicine practice that uses mainly plants for the treatment in humans (Chopra and Doiphode, 2002). Medicinal plants are well-thought as a rich resource of ingredients, which can be used in better drug development and combination. The compounds found in plants are of many kinds, but mainly they contain constituents like alkaloids, glycosides, polyphenols, and terpenes. Some plants consider as important source of nutrition while others are suggested for their beneficial values (Sakha *et al*, 2018). Ayurveda is already well accepted and used since thousands of years, ayurvedic system of medicine has its long history of healing potential. Today most of the drugs are attained from natural sources or semi synthetic derivatives of natural products which are used in the traditional systems of medicine. Thus, it is a sensible method to drug finding to screen traditional natural products as an alternative of randomly synthesized chemical moieties (Biradar *et al*, 2008). There are many ayurvedic formulations which are

claimed to be antimicrobial in nature. These preparations contain many plants in it so that the effect of the preparation becomes faster and more effective.

Bilwadi Agada mentioned in Ashtanga Hridaya chapter 36/84-85 (Vagbhatt and Hridaya, 2009) comprises the effect in versatile infective conditions such as Garavisha, Jwara, Visuchika seeing the effectiveness among various infective conditions it becomes necessary to evaluate the effects of this compound preparation against certain microbes. According to Udupa *et al* (1994) drug like Bilwa (*Aegle marmalose* Corr.) is a proven anti-inflammatory drug. It is found suitable for the treatment of pain and swelling as virtue of its Ushna Virya, Katu Vipaka and Kashaya, Tikta rasa. Bilwadi agad is mentioned in Ayurveda having thirteen ingredients, each ingredient has its own property of *krimighna*, *jantughna*, *vishghna* and *rasayan* property. So this preparation has been selected to evaluate the antimicrobial activity. In this study, an attempt has been made to evaluate the antimicrobial activity of aqueous and alcoholic extracts of *Bilwadi agad* against human pathogenic bacteria by performing well diffusion method.

Various workers reported activities of individual ingredients of Bilwadi Agada which shows potent anti-microbial as well as antifungal activities. As per Jyothi *et al* (2010), crude extracts of *Aegle marmelos* (hexane, cold methanol and hot methanol extracts at a concentration of 100 mg / ml) showed positive results especially against *E. coli*. Study done by Ramya *et al* (2012) showed that ethanolic leaf extracts of *Aegle marmelos* exhibited significant activity towards bacterial strains like *B. subtilis* and *E. coli* were more sensitive towards the treatment when compared to *S. aureus*.

Need of study

Infectious diseases seems to be a major health problem in underdeveloped countries and makes a trouble for human beings. Infections involve complicated interaction of parasite and their effects. Microbes inhibit every corner of environment, colonize skin and parts of our respiratory and gastrointestinal tracts. Despite decade of dramatic progress in their treatment and prevention, infectious diseases remains a main cause of death and debility and are responsible for worsening the living conditions of millions of people around the world. The rise and spread of antibiotic resistance, as well as the development of new disease causing agent strains, is of significant concern to the global heath community. Antimicrobials are critical in raising the global burden of infectious diseases and successful disease treatment involves the development of new drugs. In present world of emergence of multi drug resistance to human pathogenic organisms, there is constant need for development of new antimicrobial agents and antifungal agents from other sources involving plants. It is our duty to search for an ideal remedy which is safer, cost effective and easily available for our society. According to our concept of study *Bilwadi agad* can be used as an antimicrobial agent. *Bilwadi agad* mentioned in *Asthang Hridyam* chapter 36/84-85 (Gupta, 2011) and *Asthang Sangrah* chapter 42/87-88. This yog has been indicated in poisonous conditions like *sarpa, luta, vrischik mushak dansa, jwar, visuchika, ajeerana, gar visha* etc (Shastri, 1988). Present study reveals the results of content of *Bilwadi agad* as new anti-infection or antimicrobial agent. In the present study an attempt has been made to help us to understand the importance of traditional medicine in the treatment of different bacterial disease Further it may be noted that traditional/ Ayurvedic medicine shortens the length of treatment, increases patient compliance as well as reduces overdose and helps patients to avoid toxicity or other side effects.

MATERIALS AND METHODS

Collection of drug

All the ingredients of *Bilwadi agad* (100gm) were collected each in pure form from their natural habitat. The main ingredient of *Bilwadi agad* like *bilwamoola*, *sursapusphmanjari* were collected from Rishikul campus Haridwar in winter season. *Karan phal, triphala, sunthi, maricha, pippli* and *tagar* were bought from pannalal store Haridwar. *Suraha and Daru haridra* were collected from Dhanaulty in Uttarakand. *Haridra* was collected from Himachal Pradesh, 7 litres of goat urine was collected from farmer house in Haridwar. All the ingredients of *Bilwadi agad* were identified and verified by imminent experts of *Dravyaguna* Dept. at Rishikul Campus, Haridwar Uttarakand Ayurved University.

Preparation of drug

Powder of ingredients of *bilwadi agad* was prepared at Hans Pharmacy, Sidcul Haridwar. The powder of all these ingredients were mixed thoroughly, the process of *bhavana* with *ajamutra* were carried out in *Rasashastra* Department at Rishikul campus Haridwar. Total 7 bhavana was given to prepare *bilwadi agad*. The obtained *bilwadi agad* was black brownish in colour with smell of *ajamutra*.

Analytical study

Prepration of extract : Test sample: Aqueous and Alcoholic extract of *Bilwadi Agad* had been prepared by reflex extraction method.

For aqueous extraction - 20 gram of bilwadi *agad* was added to 200 ml of solvent (distilled water) and kept in reflex extractor for 1 hour. Filter it with filter paper and residue was kept in water bath until it reaches to dry form. From this process 3.6 gram dry extract was obtained.

Now from the dry extract, we took 500 mg, 1 gram, 1.5 gram of dry extract and 10 ml of water is added in each from which 5%, 10% and 15% concentration of aqueous extract of *bilwadi agad* was obtained.

For alcoholic extraction -20 gram of bilwadi *agad* was added to 200 ml of solvent (methanol) and put it in reflex extractor for 1 hour. Filter it with filter paper and residue was kept in water bath until it reaches to dry form .from this process 3 gram extract was obtained.

10 ml of methanol was added to 500 mg, 1 gram, 1.5 gram of dry extract respectively from which 5%, 10% and 15% concentration of alcoholic extract of *bilwadi agad* was obtained.

Antimicrobial study

Methods : Selection and collection of pathogens:

For the present study a total of 10 bacterial strains i.e *Escherichia coli*, *Klebsiella pneumonia, Staphylococcus aureus*, *Pseudomonas aeruginosa, Salmonella typhimurium*, *Salmonella paratyphii B, Vibrio cholerae*, *Enterobacter aerogenes, Shigella dysenteriaes, Clostridium botulinum* were selected. The pathogenic strains of different species of bacteria were procured from 'Institute of Microbial Technology' (IMTECH), Chandigarh, ATCC and the stock cultures maintenance & antibacterial study were done at 'Analytical Division of Bilwal Medchem and Research Laboratory Pvt Ltd. Jaipur (Rajasthan) with registration no. 2005/PO/RcBt/S/18/CPSEA.

Groups design

Negative control

- Distilled water
- Alcohol

Positive control

Ceftraixone (100mg/ml) for *E. coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Salmonella paratyphii B*, *Vibrio cholerae*, *Enterobacter aerogenes*, *Shigella dysenteriaes*, *Clostridium botulinum.*

Test groups

- 5 % solution of Aqueous Extract
- 10% solution of Aqueous Extract
- 15% solution of Aqueous Extract
- 5% solution of Alcoholic Extract
- 10% solution of Alcoholic Extract
- 15% solution of Alcoholic Extract

Well diffusion method was used to screen the antimicrobial activities of different solvent extracts. Muller Hilton agar solution was prepared and poured into sterile petri dishes. Upon solidification bacteria were spread in zig zag motion with cotton bud. After that, wells were made using a sterile borer (4mm in diameter) into agar plates. Then 5%, 10%, 15% of aqueous and alcoholic extract along with positive control (ceftraxione) was added to wells, respectively. The plates were incubated at 37^0C for 18 hours. Antimicrobial activity was detected by measuring the zone of inhibition appeared after the incubation period. Finally the mean of the above findings was calculated

Determination of the activity index

The activity index of the sample was calculated as

Activity index (AI) = Zone of inhibition of the sample/ Zone of inhibition obtained for standard antibiotic drug.

RESULTS

The results of various antimicrobial activity of bacterial strains are shown in Tables 1, while Table 2 shows the activity index of bacterial strains and Fig. 1 shows zone of inhibition (mm) with different bacterial strains.

Interpretation of antimicrobial study

- The Antimicrobial activity of water and alcohol extract of *Bilwadi agad* on different strains (*E. coli*, *klebsiella pneumoniae, Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Salmonella paratyphii B*, *Vibrio cholerae*, *Enterobacter aerogenes*, *Shigella dysenteries* and *Clostridium botulinum*) at different concentration 5%, 10%, 15% showed that:
- Inhabitation was absent on 5% concentration of aqueous extract of *Bilwadi agad.*
- Alcoholic extract of *bilwadi agad* at 5% concentration showed 15,12,12,11,15,16,15,17, 16,16 mm zone of inhibition and it was biologically active because the activity index of this sample found more than 0.5 except for some pathogens *viz*, (*Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella paratyphi B*, *Vibrio cholerae* and S*higella*

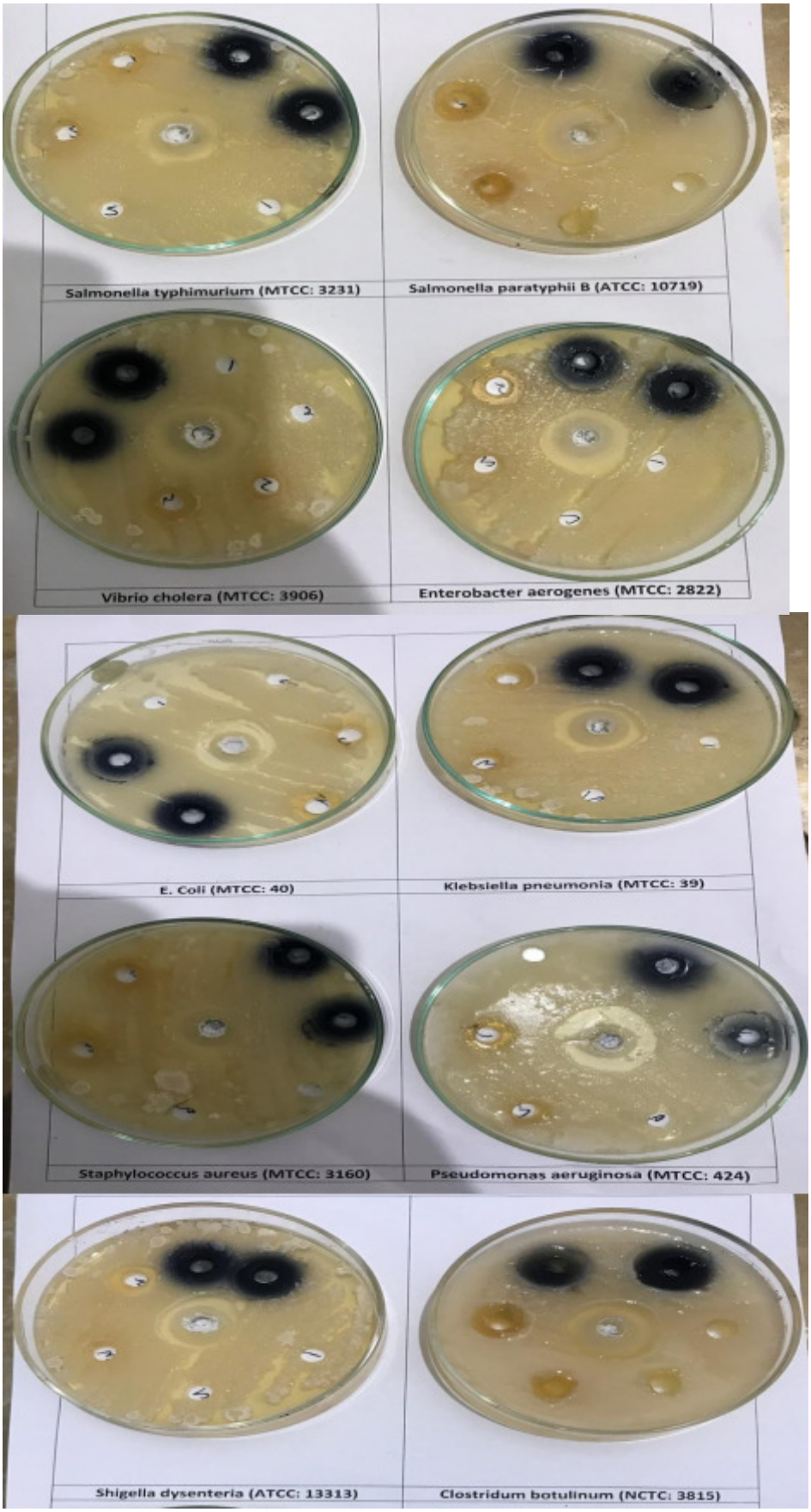

Fig. 1 : Showing Zone of Inhibition (mm) with different bacterial strains.

Fig. 1 continued...

Fig. 1 continued...

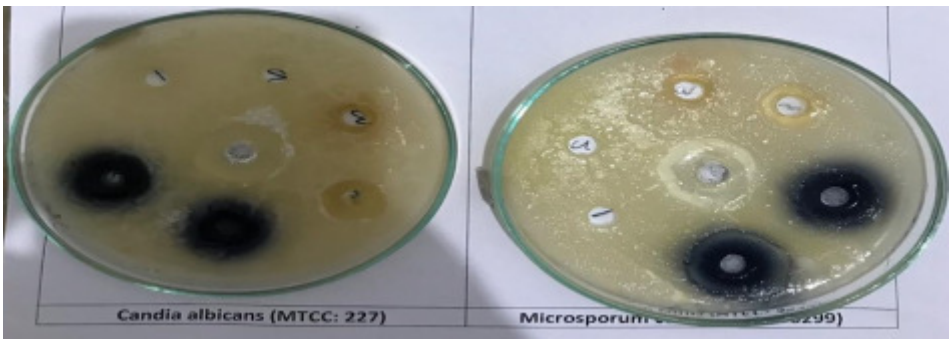

Table 1 : Antimicrobial activity of water and alcohol extract of *Bilwadi agad* on different strains

Microorganisms	-ve control		Aqueous extract			Alcoholic extract			+ve control
	Water	Alcohol	5%	10%	15%	5%	10 %	15 %	
E. coli	5	7	13	15	17	15	17	20	28
Klebsiella pneumoniae	5	6	10	12	17	12	14	18	25
Staphylococcus aureus	5	6	13	14	17	12	14	21	27
Pseudomonas aeruginosa	5	8	14	16	18	11	15	17	31
Salmonella typhimurium	5	7	12	15	16	15	17	23	27
Salmonella paratyphii B	5	6	14	15	19	14	16	20	39
Vibrio cholerae	5	8	12	14	14	11	15	20	30
Enterobacter aerogenes	5	7	11	14	15	15	17	21	25
Shigella dysenteriae	5	8	15	17	18	13	16	19	37
Clostridium botulinum	5	6	11	14	15	12	16	20	23

Table 2 : Activity Index of antimicrobial activity of water and alcohol extract of *Bilwadi agad* on different strains.

Microorganism	Aqueous extract			Alcoholic extract		
	5 %	10 %	15 %	5 %	10 %	15 %
E. coli	0.46	0.54	0.61	0.54	0.61	0.71
Klebsiella pneumoniae	0.40	0.48	0.68	0.48	0.56	0.72
Staphylococcus aureus	0.48	0.61	0.74	0.44	0.61	0.91
Pseudomonas aeruginosa	0.45	0.57	0.64	0.35	0.54	0.61
Salmonella typhimurium	0.44	0.56	0.59	0.56	0.63	0.85
Salmonella paratyphii B	0.36	0.58	0.73	0.36	0.62	0.77
Vibrio cholerae	0.40	0.47	0.47	0.37	0.50	0.67
Enterobacter aerogenes	0.44	0.56	0.60	0.60	0.68	0.84
Shigella dysenteriaes	0.41	0.71	0.75	0.35	0.67	0.79
Clostridium botulinum	0.48	0.61	0.65	0.52	0.70	0.87

dysentriae– their activity index was less than 0.5).

- Aqueous extract of *bilwadi agad* at 10% of concentration showed 15, 12,14, 16, 15, 15, 14 14, 17,14 mm zone of inhibition and biologically active against *E.coli* (0.54), *Staphylococcus aureus* (0.61), *Pseudomonas aeruginosa* (0.57), *Salmonella typhimurium* (0.56), *Salmonella paratyphi* B (0.58), *Enterobacter aerogenes* (0.56), *Shigella dysentriae* (0.71) and *Clostridium botulinum* (0.61) and non active against *Klebsiella pneumonie* and *Vibrio cholerae*.
- Alcoholic extract of *bilwadi agad* at 10% concentration showed 17, 14, 14, 15, 17, 16,1 5, 16, 15, 16,17,17 mm zone of inhibition. The activity index of *E.coli* (0.61), *Staphylococcus aureus* (0.56), *Pseudomonas aeruginosa* (0.54), *Klebsiella pneumoniae* (0.56), *Vibrio cholerae*(0.50), *Salmonella typhimurium* (0.63), *Salmonella paratyphi B* (0.62), *Enterobacter aerogenes* (0.68), *Shigella*

dysentriae (0.67) and *Clostridium botulinum* (0.70), which means alcoholic extract with 10% concentration was biologically active against all the microbes.

- Aqueous extract of *Bilwadi agad* at 15% concentration showed 17, 17, 17, 18, 16, 19, 14, 15, 18,15 mm zone of inhibition. The activity index of *E.coli* (0.61), *Staphylococcus aureus* (0.74), *Pseudomonas aeruginosa* (0.64), *Klebsiella pneumonia* (0.68), *Salmonella typhimurium* (0.59), *Salmonella paratyphii B*(0.73), *Enterobacter aerogenes*(0.60), *Shigella dysentriaes* (0.75) and *Clostridium botulinum* (0.65) hence *Bilwadi agad* at 15% aqueous extract was found biologically active against all pathogens except *Vibrio cholerae*(0.47).
- Alcoholic extract of *bilwadi agad* at 15% concentration showed 20, 18, 21, 17,23, 20, 21, 21, 19, 20 mm zone of inhibition with activity index of *E.coli* (0.71), *staphylococcus aureus* (0.56), *Pseudomonas aeruginosa* (0.61), *Klebsiella pneumoniae* (0.72), *Vibrio cholerae*(0.67), S*almonella typhimurium*(0.85), *Salmonella paratyphii B*(0.77), *Enterobacter aerogenes* (0.84), *Shigella dysentriaes* (0.79) and *Clostridium botulinum*(0.87). Hence, *Bilwadi agad* with 15% alcoholic concentration showed significant result.

DISCUSSION

Literay review of bilwadi *agad* showed potent antimicrobial activity, each ingredients of bilwadi agad have antimicrobial properties. *Aegle marmelos* marked antibacterial properties, shows its effect against *Staphylococcus aureus* (Rahman, & Parvin, 2014). *Berberis aristate* showed antimicrobial activity against different bacterial strains like *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa, Staphylococcus pneumonia* (Mazumder *et al*, 2011). Similarly *Pongamia pinnata* (Yadav *et al*, 2011), *Cedrus deodara* (Kumar *et al*, 2014), *Terminalia chebula* (Gupta *et al*, 2010), *Valeriana wallichii* (https:// www.bimbima.com), *Terminalia bellirica* (Deb *et al*, 2016), *Emblica officinalis* (Sharma *et al*, 2017), *Piper longum* (Lokhande *et al*, 2007), *Piper nigrum* (Karsha and Lakshmi, 2010), *Zinger officinalis* (Nafiseh *et al*, 2013), *Ocimum sanctum* (Hanna *et al*, 2016) are antimicrobial in nature. *Curcuma longa Linn* has antioxidant, immune modulatory effect*, has kusthghna, vishahar, jantughna properties* (Sason *et al*, 2016). On the other hand, *Ajamutr*a also exhibits as antimicrobial agent (Tomar *et al*, 2018). Another study reveales that *bilwadi agad* showed mild to moderate antimicrobial activity against *Basilus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella parathphii B* (Binorkar and Sreekrishnan, 2013). Majority of the drugs of *bilwadi agad* are *tikta* (bitter), *katu* (pungent) *rasa pradhan* which acts as *kapha-vatahara* (pacifies *kapha* & *vata*). All drugs are *ushna veerya* (hot potency), majority are *katu vipaka* hence can act as *vishgna* and *keetaghna.*

CONCLUSION

In this study 5,10 and 15% concentration of aqueous and alcoholic extract of bilwadi agad was evaluated for its antimicrobial activity against following human pathogenic strains *viz*., *E. coli, Klebsiella pneumoniae, Staphylococcus aureus*, *Pseudomonas aeruginosa, Salmonella typhimurium*, *Salmonella paratyphii B*, *Vibrio cholerae*, *Enterobacter aerogenes, Shigella dysenteriaes*, *Clostridium botulinum.* Both type of extract of *bilwadi agad* have shown a dose dependent antimicrobial effect. Alcoholic extract of *bilwadi agad* have shown better result in comparison to aqueous extract. Aqueous extract with 10% concentration have shown positive result against all microbes except *Klebsiella pneumoniae* and *Vibrio cholerae,* while 15% concentration of aqueous extract of *bilwadi agad* have shown positive results against all the microbes used in this study. Alcoholic extract with 10% and 15% concentration of *bilwadi agad* have shown positive result against all the microbes. This study may be a boon for human kind for curing infectious disease. So we can use *Bilwadi agad* as ayurvedic antibiotic. The present study justify the use of *bilwadi agad* to treat various infectious disease caused by the microbes. This study will help us to understand the importance of traditional medicine in the treatment of different bacterial disease.

REFERENCES

Binorkar S V and Sreekrishnan C M (2013) Evaluation of 'Dashanga Agada' (Herbo-Mineral Compound) in the Management of Scorpion Sting Envenomation. *Asian J. Pharmaceut. Res. Develop.* 126-133.

Biradar Y S, Jagatap S, Khandelwal K R and Singhania S S (2008) Exploring of antimicrobial activity of Triphala Mashi—An ayurvedic formulation. *Evidence-based Complementary and Alternative Med.* **5**(1), 107-113. https://dx.doi.org/10.1093/ecam/nem002

Chopra A and Doiphode V V (2002) Ayurvedic medicine: Core concept therauptic principles and current relevance. *Medical Clinics of North America* **86**(1), 75-89. vii http//dx.doi.org/10.1016/S0025-7125(03)00073-7.

Deb A, Barua S and Das B (2016) Pharmacological activities of Baheda (*Terminalia bellerica*): a review. *J. Pharmacog. Phytochem.* **5**(1), 194-197.

Gupta Kaviraj Atridev (2011) *Ashtanga Haridyam*. 11th edition, Chapter 36/84-85. Chaukhamba Krishnadas Academy, ISBN:9788121801990.

Gupta A, Mishra A K, Bansal P, Singh R, Kumar S and Gupta V (2010) Phytochemistry and Pharmacological activities of Haritaki-A review. *J. Pharm. Res*. **3**(2), 417-424.

Hanaa A, Yamani Edwin C Pang, Nitin Mantri and Margaret A Deighton (2016) Antimicrobial Activity of Tulsi (*Ocimum tenuiflorum*) Essential oil and their Major Constituents against Three Species of Bacteria. *Front. Microbiol*. **7**, 681.https://dx.doi.org/10.3389/fmicb.2016.00681

https://www.bimbima.com/herbs/tagar-valerian-uses-benefits-dosage-and-side-effects/72/

Jyothi K S and Rao B S (2010) Antibacterial activity of extracts from Aegle marmelos against standard pathogenic bacterial strains. *Int. J. Pharmtech Res.* **2**(3), 1824-1826.

Karsha P V and Lakshmi O B (2010) Antibacterial activity of black pepper (*Piper nigrum* Linn.) with special reference to its mode of action on bacteria. **1**(2), 213-215.

Kumar M, Kumar P, Kumar N, Singh A N, Lata M, Pandey A K and Singh Anurag (2014) Antimicrobial Activity of *Cedrus deodara* Linn. and *Hemidesmus indicus* Linn. Plants against clinically important micro-organism. Am. J. Phytomed. Clin. Therapeutics **2**(8), 952-956.

Lokhande P D, Gawai K R, Kodam K M, Kuchekar B S, Chabukswar A R and Jagdale S C (2007) Antibacterial activity of extracts of *Piper longum*. *J Pharm Toxicol.* **2**(6), 574-579.http://dx.doi.org/10.3923/jpt.2007.574.579

Mazumder P M, Das S, Das S and Das M K (2011) Phyto-pharmacology of *Berberis aristata* DC: a review. J. Drug Delivery and Therapeutics **1**(2), 46-50. https://doi.org/10.22270/jddt.v1i2.34

Nafiseh Shokri Mashhadi, Reza Ghiasvand, Gholamreza Askari, Mitra Hariri, Leila Darvishi and Mohammad Reza Mofid (2013) Anti-Oxidative and Anti-Inflammatory Effects of Ginger in Health and Physical Activity: Review of Current evidence. Int. J. Prevent. Med. **Suppl. 1**, S36-S42.

Nayan R Bhalodia and Shukla V J (2011) Antibacterial and antifungal activities from leaf extracts of *Cassia fistula* l.: An ethnomedicinal plant. *J. Adv. Pharmaceut. Tech. Res*. **2**(2), 104-109. https://dx.doi.org/10.4103/2231-4040.82956.

Rahman S and Parvin R (2014) Therapeutic potential of *Aegle marmelos* (L.)-An overview. *Asian Pac. J. Trop. Dis.* **4**(1), 71-77.https://dx.doi.org/10.1016/S2222-1808(14)60318-2

Ramya S, Jayakumararaj R, Krishnasamy G, Periathambi N and Devaraj A (2012) Antibacterial activity of ethanolic leaf extracts of *Aegle marmelos* (l) Corr. *Int. Res. J. Pharmaceut. and Appl. Sci.* **2**(6), 74-79.

Sakha H, Hora R, Shrestha S, Acharya S, Dhakal D, Thapaliya S and Prajapati K (2018) Antimicrobial Activity of Ethanolic Extract of Medicinal plants against Human Pathogenic Bacteria. *Tribhuvan Univ. J. Microbiol.* **5**, 1-6. https://doi.org/10.3126/tujm.v5i0.22292

Sason Rajveer, Sharma Anita and Kushwaha Ravi Kumar (2016) Conceptual study on anti toxic effect of Bilwadi agada. (A.H.U.36/84-85). *World J. Pharmaceut. Life Sci*. **2**(4), 464-470.

Sharma Kavitha, Preeti Sharma, Rashmi Srivastav, Akhilesh Srivastava and Sharma Dalip (2017) Pharmacological Study of Amalaki with Special Reference to its antimicrobial action. *Int. J. Ayurveda and Pharma Res*. **5**(5), 93-96.

Shastri Pandit Lalchandra (1988) Acaharya vagbhata ashtanga sangraha, edited by Vaidya Pandit Lalchand. Ist edition, Baidyanath ayurved publication, Nagpur; Uttersthan, page no. 785.

Tomar V, Nigam R, Pandey V, Singh A P, Roy D, Sharma A and Pal A (2018) Evaluation of *in vitro* Anti-microbial activity of Goat Urine Peptides. *J. Anim. Res.* **8**(1), 33-37.

Udupa S L, Udupa A L and Kulkarni D R (1994) Studies on the anti-inflammatory and wound healing properties of *Moringa oleifera* and *Aegle marmelos*. *Fitoterapia* **65**(2), 119-123.

Vagbhatt S and Hridaya A (2009) With commentaries Sarvangasundara of Arundatta & Ayurvedarasayana of Hemadri, Chikitsa Sthana 8/55-56. *Chaukhambha Sanskrit Sansthan, Varanasi.*

Yadav R D, Jain S K, Alok S, Prajapati S K and Verma A (2011) Pongamia pinnata: an overview. *Int. J. Pharmaceut. Sci. Res.* **2**(3), 494-500. http://dx.doi.org/10.13040/IJPSR.0975-8232.2(3).494-00.